PROCEEDINGS.

VOL. XXVII. DECEMBER, 1929. No. 3.

## Missouri Branch.

*Washington University School of Medicine, November 13, 1929.*

4657

### The Effect of Pitressin (Vaso-pressin) upon the Heart.

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It has been pointed out by one of us[1] that pitressin (Vaso-pressin) caused a marked drop in blood pressure and decreased pulse rate in the unanesthetized dog. The decreased pulse rate we then believed to be of vagal origin. The present work is a continuation of these earlier experiments.

The effect of the drug upon the heart was noted by recording the electrical potential changes in the action current by the string galvanometer in leads I and III, although usually only the latter lead was employed. Unanesthetized dogs were used. The pitressin was injected intravenously in small doses 0.1 to 0.5 cc. and in some cases followed by the injection of atropine sulphate in doses of 0.2 mgm. or more per kilo. In other experiments the atropine was injected intravenously before the pitressin and other experiments were performed on animals in which the vagi had been severed under ether anesthesia a sufficient time earlier for the animals to have recovered.

In a series of unanesthetized dogs the effect of pitressin upon the blood pressure and pulse rate was studied by means either of a mercury monometer or of a membrane monometer. The pulse rate was counted before, during and following the fall in blood pressure. Other conditions were similar to the above described experiments.

[1] Gruber, *J. Pharm. Exp. Therap.*, 1929, xxxvi, 155.

The effect of pitressin upon coronary flow was studied in excised perfused rabbit's hearts.

*Results.* The electrocardiographic studies in the normal unanesthetized dog shows the heart slowed, 10 to 15 seconds after the injection of pitressin. This slowing was quickly followed by an acceleration which was subsequently followed by marked slowing of the heart, which persisted for several minutes. During the period of excessive slowing high branching of the T-wave occurred with a marked increase in the height of the wave. In some animals in which the T-wave appeared inverted in the control electrocardiogram it was changed to an upright T-wave after pitressin administration. If atropine in doses sufficient to paralyze the vagi is injected in such animals partial heart block is noted, *i. e.,* P-waves appear in the record without ventricular complexes. In most instances the P-waves following such treatment are inverted or diphasic in character and remain so even though atropine tachycardia is established.

An injection of atropine before the injection of pitressin did not prevent the slowing of the heart completely. Neither did the fact that the vagi had been cut. In such hearts pitressin appears very much more toxic. In all animals high T-waves were observed. In some high branching of the T-waves, shifting pace-maker, nodal rhythm, many ventricular extra systoles, paroxysmal ventricular tachycardia and even ventricular fibrillation were encountered.

The blood pressure records as well as the electrocardiogram of the heart beat show the slowing of the heart beat to be mainly of central origin as it disappears quickly after the injection of atropine sulphate and does not occur to the same degree in animals in which the vagi are cut or paralyzed by atropine. Likewise the acceleration disappears to a large extent by either cutting the vagi or administrating atropine. The acceleration occurs during the fall in blood pressure and is mainly, though not wholly, a compensatory phenomenon, an attempt on the part of the body to maintain a normal blood pressure.

The coronary vessels of rabbits constrict when exposed to pitressin. Moderately high concentration of pitressin injected into the perfusate, suddenly slows the perfusion flow, requiring considerable time for its recovery.

Most of the electrocardiographic changes noted, especially the high T and high branching of the T-waves, we believe can be accounted for by the asphyxia of the heart muscle and accumulation of products of metabolism through coronary vaso-constriction.

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## The Respiration in Plasma in Disease Due to Filtrable Virus.

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(Introduced by McKim Marriott.)

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In 1927 Kempner,[1] working in Warburg's Institute, demonstrated the disappearance of oxygen and the development of $CO_2$ in the plasma of chickens suffering from fowl-plague. He obtained the plasma by adding heparin to whole blood taken at the height of the disease. The plasma was placed in Warburg's manometric apparatus, saturated with a mixture of $CO_2$ and $O_2$ and kept at constant temperature. Readings were done for several hours and changes in pressure noted. Since the plasma from normal chickens did not show respiration and the changes observed were sufficiently great to be outside of experimental error, Kempner concluded that the respiration was connected with the metabolism of the virus.

Using Warburg's apparatus and the technic described above, we repeated the experiments using plasma obtained from pigs injected with hog-cholera. It was impossible to obtain virus of fowl-plague either in the United States or in Canada. Plasma from cases of measles was similarly used as there is considerable evidence for its being a virus disease. In addition plasma from miscellaneous affections, including rheumatic fever, was similarly studied.

Our results were as follows: (a) Experiments with normal human or animal plasma did not show a disappearance of oxygen. (b) A suspension of red cells from chickens was similarly used. Respiration was measured and found to occur in agreement with the values obtained by Warburg[2] for goose-blood cells. (c) Hog cholera plasma was obtained from experimentally infected hogs at the height of the disease. The animals were killed by cutting their throats and the blood caught directly in large sterile test-tubes containing heparin. Autopsy in each case showed that the animal had been suffering from hog cholera. Blood from 15 hogs was used and in most instances duplicate experiments were done. No disappearance of oxygen or appearance of carbon dioxide was noted and under anaerobic conditions no glycolysis took place. (d) An epidemic of measles during the spring of 1928 furnished the material

[1] Kempner, W., *Klin. Woshenschr.*, 1927, vi, 2386.

[2] Warburg, O., *Über den Stoffwechsel der Tumoren*, 1926, F. Springer, Berlin, p. 199.

for the study. The blood was obtained either in the pre-eruptive state when Koplik spots were present or during the early eruptive stage. Bacteriological culture of the blood on various media recommended for the growth of organisms from measles, showed no growth. Plasma from 15 cases was used and no changes in pressure under either aerobic or anaerobic conditions were noted. (e) Several experiments were done with plasma obtained from miscellaneous diseases of doubtful etiology and among them were 5 cases of rheumatic fever. Negative results were again obtained and bacteriological culture showed no organisms.

In none of the experiments were the changes greater than those due to experimental error.

We conclude that in our experiment respiration as measured by Warburg's method is not a general finding in plasma infected with filtrable viruses and that the virus character of the disease cannot be demonstrated by this method.

We acknowledge our indebtedness to Dr. Ethel Ronzoni for her great assistance and we are also indebted to Dr. Durant of the University of Missouri and Dr. A. E. Bott, of the Corn Belt Serum Company, East St. Louis, Illinois, for enabling us to get the hog cholera plasma.

## 4659

### Studies in Carbohydrate Utilization by Organisms of the Genus Mycobacterium.

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By means of direct quantitative carbohydrate determinations employing the Shaffer-Hartmann blood sugar method I have shown there is a rather wide utilization of carbohydrates by organisms of the genus Mycobacterium.

The reaction changes both in plain broth and carbohydrate broth cultures of the organisms of this genus are toward progressive increase in alkalinity. The reaction change is less rapid in the presence of utilizable carbohydrates. This increase in alkalinity whether in plain broth or carbohydrate broth cultures has been shown to be associated with an increase in the ammonia content of the media. This increase in ammonia is in most cases approximately equivalent

to the increase in the titrable alkalinity. In other cases other substances either acid or alkaline in character, which are apparently derived from protein cleavage, are involved to some extent in the causation of the reaction changes and changes in titratable alkalinity. Such changes are accounted for by the ammonia increase equally as well in the carbohydrate broth cultures as in the plain broth cultures.

The ammonia production was found to be distinctly less for the production of a given amount of growth in the presence of utilizable carbohydrates. This diminished ammonia production was associated with a diminution in the degree of reaction change of the media. Also the higher the carbohydrate concentration, so long as it is within the limits that will permit growth, the less the ammonia increase and the less the reaction change of the media during the production of a constant amount of growth. Thus not only the presence, but also the concentration of a carbohydrate, is a factor determining the degree of protein sparing action it exerts.

The presence of utilizable carbohydrate does not directly have any effect upon the reaction change of the media. Utilizable carbohydrates apparently affect the reaction changes only to the extent that they alter protein cleavage by the organisms. Thus there is no accumulation of cleavage products of the carbohydrates that in any way alters the pH of the medium. This contrasts strikingly with the carbohydrate utilization by most bacteria belonging to other genera.

The utilization of carbohydrates by the organisms of the group studied is characterized by a gradual decrease in the carbohydrate contained in the media until it completely disappears. There is no inhibition of the growth of the organisms at any time by products of the carbohydrate cleavage, such as is the case in the majority of organisms, the carbohydrate utilization of which has been studied.

The $CO_2$ produced has been shown to vary directly as the growth of the organisms in carbohydrate-containing as well as carbohydrate-free media. The $CO_2$ produced also varies directly as the carbohydrate utilized in the carbohydrate-containing media, and in most cases more carbon dioxide was recovered than could have been derived from the carbohydrate disappearing. This may be compared to a 10% to 18% yield of possible carbon dioxide recovered from cultures of acid-producing organisms as *B. coli* and *Staphylococcus aureus*. Far more carbon dioxide was found to be liberated per mg. of ammonia produced in the presence than in the absence of utilizable carbohydrates.

No organic acid cleavage products of the carbohydrate utilization could be demonstrated either in media with varying concentrations

of the carbohydrates or in the presence of varying quantities of available oxygen.

These organisms could not be grown under anaerobic conditions. Cultures grown in limited oxygen supply grew until all the molecular oxygen was used and no further growth, carbohydrate utilization, or reaction changes of the media were demonstrable even after 2 months additional incubation. Carbohydrate utilization thus apparently takes place only in the presence of molecular oxygen.

The explanation suggested in the preliminary report,[1] namely that if the carbohydrate molecule is attacked at all it is oxidized completely to carbon dioxide and water, without any intermediate products appearing in the media, explains all phenomena observed relative to carbohydrate utilization by these organisms.

## 4660

### Effects of Combined Administration of Extracts of Anterior Lobe of Pituitary and of Potassium Iodide on Thyroid Gland.

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In former investigations from this laboratory Loeb,[1] Gray[2] and Rabinovitch[3] have shown that potassium iodide may exert a stimulating effect on the thyroid gland of the guinea pig. It does not prevent compensatory hypertrophy, but may modify its character; in the normal gland it may increase the mitotic activity as much as 40-60 times, and quite commonly increases it 20 times. Furthermore, it produces a slight increase in the size of the acinus cells and a slight softening of the colloid and a very marked increase in the number of phagocytes in the colloid. Loeb[4] has shown that oral administration of anterior pituitary substance (Armour & Co.) prevents compensatory hypertrophy of the thyroid gland and McCordock[5] has shown that it prevents the hyperplasia caused by potassium iodide. However, if instead of oral administration of anterior

[1] Merrill, Malcolm H., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 574.

[1] Loeb, Leo, *The Am. J. Surg.*, New Series, 1929, vii, 12. Endocrinol., *Bull. of Assoc. for the Study of Internal Secretion*, 1929, xiii, 1.

[2] Gray, S. H., *Am. J. Pathol.*, 1929, v.

[3] Rabinovitch, Jacob, *Am. J. Pathol.*, 1928, iv.

[4] Loeb, Leo, *J. Med. Res.*, 1920, xli, 481; *Am. J. Pathol.*, 1929, v, 71.

[5] McCordock, H. A., *Am. J. Path.*, 1929, v.

pituitary we give daily subcutaneous injections of either acid or alkaline extracts of anterior pituitary, no inhibition of the thyroid gland results, but, on the contrary, a very pronounced stimulation (Loeb and Bassett[6]). Hyperplasia and hypertrophy of the acinar epithelium, liquefaction and absorption of the colloid proceed very rapidly. After 7 daily injections of such extracts extreme changes are found in the gland resembling in many respects those obtained in typical cases of Graves disease.

We have then in potassium iodide and in acid or alkaline extract of anterior pituitary gland 2 substances which stimulate the thyroid gland of the guinea pig and which produce a very pronounced cell proliferation in the epithelium of the acini. They differ, especially, in that the hypertrophy and softening of the colloid caused by KI is very slight as compared with the pronounced effects of anterior pituitary extracts and secondly, in that, under the influence of anterior pituitary extract, the colloid is rapidly absorbed from the acini, whereas under the influence of KI it is only slightly softened and largely retained in the acini. As the result of this retention ultimately pressure may be exerted on the acinar epithelium.

Under these conditions it was of interest to determine the effect of the combined administration of KI and of extracts of anterior pituitary. Three possibilities existed as to the effects of the combined action: (1) That these 2 substances might reenforce their stimulating action; thus a summation of effects would take place. (2) KI might prevent the hypertrophic and other changes produced by anterior pituitary; this would be in accordance with the view held by a number of investigators that KI tends to produce a resting condition in the thyroid gland, and (3) both substances might tend to produce their characteristic effects and thus a competition between the 2 types of changes would result from the simultaneous administration of these 2 substances.

Our experiments have shown so far that the third possibility is realized. Seven young male guinea pigs were fed daily for a period of 17 days with 0.05 gm. KI, and seven other guinea pigs with 0.1 gm. KI. From the 10th to the 17th day 5 animals in each group (10 altogether) were injected intraperitoneally daily with 1 cc. of acid extract of anterior pituitary substance. Four control guinea pigs received only KI for a period of 17 days and 5 other control guinea pigs received for a period of 7 days injections of acid extract of anterior pituitary.

[6] Loeb, Leo, and Bassett, R. B., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 860.

The microscopic examination of these thyroid glands confirmed the results of the previous investigators as to behavior of the control animals. We made some approximate determinations of the number of mitoses in the thyroids of all animals and found, as was to be expected, a great increase in mitotic activity, namely, an average of approximately 3000 in the KI controls, and of 8000 in the controls injected with the extract of anterior pituitary. The actual number of mitoses may have been somewhat greater, as our counting aimed only at an approximate determination; the other changes observed also correspond to those described previously. In the guinea pigs treated with a combination of these 2 substances we found effects characteristic of KI as well as of acid extract of anterior pituitary. In some places we found the picture characteristic of anterior pituitary extract, namely, very hypertrophic acinus cells, absorption of the greater part of the colloid, irregular form of the acini, the lumen of which often had the shape of slits. In other parts, changes were those seen after KI feeding: a considerable increase in mitoses, a slight increase in the size of the cells, and a slight softening of the colloid; while the colloid in a number of acini showed definite softening in these cases, other acini showed rather solid colloid without diminution in the quantity of this substance. There were many phagocytes present. In still other areas we found intermediate conditions between those characteristic of KI and of anterior pituitary extract. The acinus cells were higher than those seen in KI feeding, but not quite as high as in animals injected with anterior pituitary extract. The colloid was softened. In many acini the colloid took up much fluid. Thus the lumina of these acini increased considerably; they extended considerably and, secondarily, the pressure thus exerted upon the acinus cells led again to a flattening of the acinus cells and reduced probably the mitotic activity. This increase in fluid colloid in the acini and the pressure resulting therefrom caused in many cases a breaking through of the walls separating adjoining acini and thus there developed large irregular compound acini, into the lumen of which spurs, the remnants of broken through walls, protruded. There were considerable areas in which these enlarged acini were found. It is probable that the pressure thus secondarily exerted upon the acini by the swollen and liquefying colloid tended to diminish somewhat the number of mitoses; it was, on the average, 2500, therefore about 18 times more than is found in normal glands. In the thyroids of guinea pigs injected with anterior pituitary extract the number of phagocytes is increased, but not to the same extent as in KI guinea pigs. Similarly

in the animals treated with both substances the number of phagocytes is increased without being as frequent as in KI animals. Not only do we find different appearances in different parts of the same gland, but there are also differences in the glands of different animals as to the prevalence of one or the other type of reaction.

Characteristic of the guinea pigs subjected to the combined action of these 2 substances is, therefore, a combination of the effects of both potassium iodide and of anterior pituitary extract. This leads to a variegated appearance of these glands, areas with very dilated acini and very watery colorless colloid alternating with areas found in the KI gland and with other places in which the hypertrophy and the absorption of colloid are very pronounced. On the whole, the number of acini in which a dilatation has taken place is greater in these glands than in those of either the animals treated with potassium iodide or with anterior pituitary extract. This is probably due to a combination of the greater liquefaction which takes place under the influence of anterior pituitary extract and of the retention of the softened material in the acini for which the KI is responsible.

*Conclusion:* Potassium iodide and anterior pituitary extract each exerts thus its specific effects on the thyroid gland under the conditions of our experiments; no real summation, but a mosaic of areas in which one or the other effect predominates, is the result of these combinations. It is probable that the mechanism by which KI and anterior pituitary extract cause the great increase in growth processes in the epithelial tissues of the thyroid gland, is different in the case of both these substances; thus a simple summation does not take place. It appears, furthermore, that under the conditions of our experiment in which considerable quantities of KI were fed to the guinea pigs previous to the series of injections of anterior pituitary extract, and in which thus KI has an advantage over the latter substance, potassium iodide diminishes somewhat the reactivity of the thyroid gland to the effects of anterior pituitary extract. It is possible that the latter effect is mainly due to the greater retention of colloid in the acini of animals fed with KI.

In further investigations, which are being conducted at the present time, we intend to determine the effect of the combination of these two substances on the one hand, when they are administered simultaneously from the beginning and, on the other hand, when the first period in which KI alone is administered is still further increased.

4661

## The Electrocardiographic Changes in Anoxemia.

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A sign usually considered to be pathognomonic of coronary thrombosis is the high branching of the "T" wave of the electrocardiogram. It appears soon after the accident of coronary occlusion and reaches its maximum intensity within a few hours; and disappears after a few days. Although the mechanism of the change is unknown, it has been attributed to a number of factors, among which may be mentioned the influence of the current of injury on the normal change of electric potential of the heart,[1] anoxemia of the heart muscle[2] and asphyxia.

The following experiments enable us to draw certain conclusions concerning the mechanism of the change.

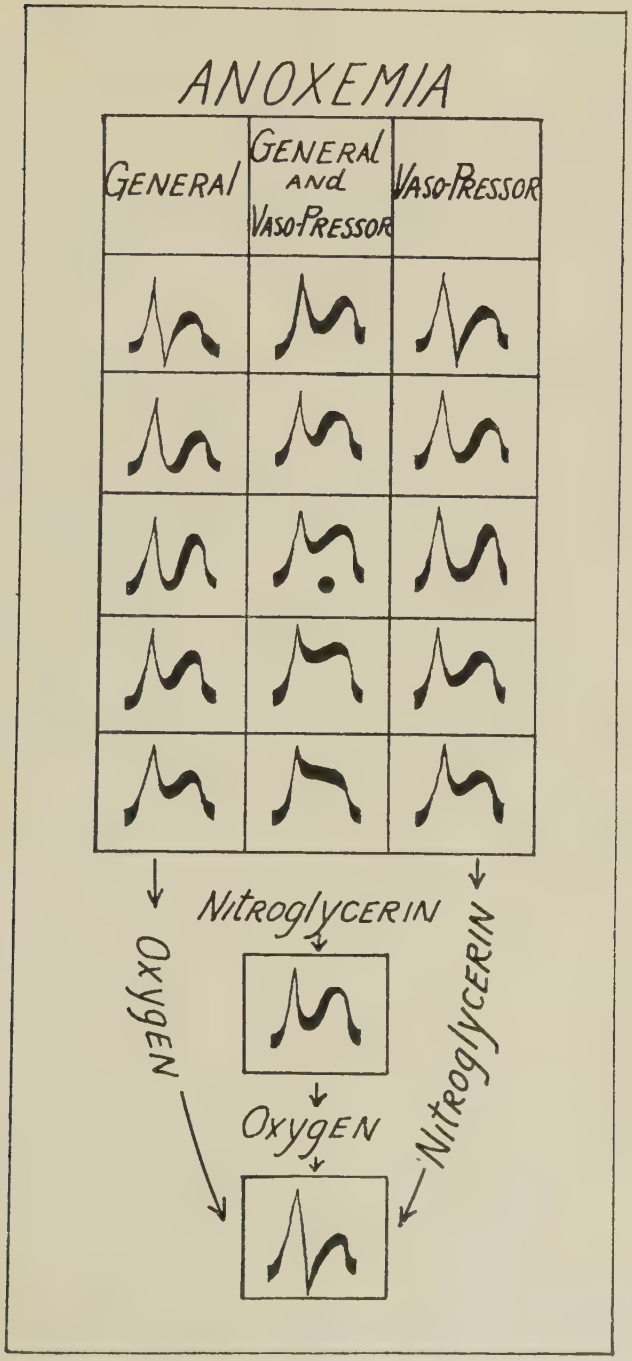
Dogs were anesthetized with amytal and were placed under conditions of anoxemia. To accomplish this, they were connected by means of a tracheal cannula to a large dead space into which the animal rebreathed and from which the carbon dioxide was absorbed with soda lime. They, therefore, breathing air from which the oxygen was gradually reduced, passed into a state of anoxemia, and carried to a point of complete asphyxia at which time the oxygen content of the arterial blood was as low as 20%. When the oxygen saturation of the arterial blood fell below 50% of the normal, the animals showed in their electrocardiogram changes similar to those observed clinically in coronary occlusion. The release of the animal from the anoxemia state caused the electrocardiogram to return to normal.

As was pointed out by Gruber and Kountz,[3] a picture similar to anoxemia was produced by the injection of pitressin in normal unanesthetized animals. They have also shown that one of the primary actions of the drug on the heart was that of coronary constriction. The change in the electrocardiogram produced by the drug could be caused to disappear by vasodilator substances such as sodium nitrite. Therefore, as the action of pitressin is that of coro-

[1] Clark, N. E., and Smith, F. J., *J. Lab. and Clin. Med.*, 1925, xi, 1071.

[2] Katz, L. N., Feil, H. S., and Scott, R. W., *Am. Heart J.*, 1929, v, 77.

[3] Gruber, Chas. M., and Kountz, Wm. B., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvii, 161.


ANOXEMIA
GENERAL
GENERAL AND VASO-PRESSOR
VASO-PRESSOR
NITROGLYCERIN
OXYGEN
OXYGEN
NITROGLYCERIN

nary constriction the result may be explained on the basis of anoxemia of the heart.

A combination of the two experimental procedures, namely the giving of pitressin to anoxemic animals, produces a change similar to that seen in the most extreme changes of coronary occlusion. In the curves under this condition, it was found in the electrocardiogram that the "T" wave branched directly from the downstroke of the "QRS" complex. The electrocardiogram immediately returned to normal if oxygen and vasodilator substances were given.

The change in the electrocardiogram began by a decrease in amplitude of the "T" wave followed by inversion, which occurred at 30% of oxygen unsaturation of the arterial blood. As the anoxemia progressed, however, the "T" wave became upright, increased in height and widened at the base. The widening occurred at the expense of the S.T. interval which became short and was finally obliterated. Further widening of the base of the "T" wave as occurred when pitressin was given to animals in a state of anoxemia, causes the crest of the "T" to fuse with the "QRS" downstroke.

The conclusion is that anoxemia of the heart muscle may be the cause of the pathognomonic sign seen in the electrocardiogram. Anoxemia occurs as a result of shutting off of blood. This electrical phenomena disappears experimentally by relief of the coronary constriction or with the administration of oxygen. Clinically, in coronary thrombosis one might assume that its disappearance is due to the establishment of a collateral circulation.

The study suggests the possibility of a clinical test to determine the efficiency of the coronary circulation.

# Iowa Branch.

*State University of Iowa, November 7, 1929.*

## 4662

### The Complexity of the Achilles and Patellar Tendon Reflex Arcs.

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The concept that the highest neurological levels are functional parts of the peripheral arcs is becoming more and more widely accepted. Of special importance for this concept is the work of Hoffmann,[1] Travis and Dorsey,[2, 3] Herren and Haterius,[4] and Coghill.[5] Hoffmann and Travis and Dorsey found that both anatomical and physiological alterations of the higher nerve centers alter reflex time in humans. Herren and Haterius found that functional changes in the higher nerve centers of the rat produce changes in the reflex time. All of these workers used the action current technique for measuring the reflex response latency. Further experiments in which this same technique was utilized furnish additional evidence for the proposition that the highest levels of irradiation are functional parts of the lowest levels of irradiation.

In the first study patellar reflex records were secured on 122 and Achilles reflex records on 119 normal adults. The reflex time in both reflexes regularly correlates higher with standing height than with measures that should more closely approximate the true lengths of the peripheral arcs.

A second study shows that during severe tonic blocks in stuttering the patellar reflex time is reduced, whereas in the normal speech of both stutterers and normal speakers it is not affected. The reductions in 8 severe cases varied from 10 to 50% of the reflex time

[1] Hoffmann, F. A., *Deutsch. Arch. f. Klin. Med.*, 1916, cxx, 173.

[2] Travis, L. E., and Dorsey, J. M., *Arch. Neurol. and Psychiat.*, 1929, xxi, 613.

[3] Travis, L. E., and Dorsey, J. M., *Arch. Neurol. and Psychiat.*, 1929, xxii, 99.

[4] Herren, R. Y., and Haterius, H. O., Proc. Soc. Exp. Biol. and Med., 1929, xxvi, 657.

[5] Coghill, G. E., *Arch. Neurol. and Psychiat.*, 1929, xxi, 989.

during silence. There appears to be some relationship between the severity of the block and the amount of reduction of the reflex time.

A third study shows that alcohol decreases the patellar reflex time in dogs. In general each of 5 dogs ranging in weights from 31 to 37 pounds was given intravenously rapidly 20 cc. of 97% alcohol in 80 cc. of normal salt solution. The reflex times of each dog were consistently but irregularly shortened after the alcohol injection. In addition the ranges of reflex time were greater. The reductions in reflex time varied from 6 to 30% of that of the normal period.

All of these findings indicate that the nerve impulse in these reflexes may travel beyond the cord to certain higher nerve centers or at least that the higher centers are a functional part of the lower and that an alteration of the functions of the former alters prejudicially the functions of the latter.

4663

## The Effect of Submersion in Water at Various Temperatures On Respiration.

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The data herein presented include only a part of an investigation of the disturbances in respiration due to submersion in water of various temperatures.

Wasserman,[1] Bazett,[2] and Hill and Flack[3] made observations on the effect of bath temperature changes on respiration, but we have failed to find a complete picture, beginning with cold and ending with hot water. However, the results reported by these investigators parallel our findings.

In securing the data, use was made of the constant temperature baths provided by the Psychopathic Hospital. Each subject was placed in the tub in a prone position during which time a record of normal respiration was taken. Following this the tub was filled, temperature changes made and data recorded as indicated by Fig. 1. Time is recorded in 5 sec. intervals.

[1] Wasserman, Max., *Casopis ceskych lekaru*, 1924, Nos. 16, 19. (Quoted from *Archiv. des Maladies, du coeur des Vaisseax et du Sang.*, 1926, 50.)

[2] Bazett, H. C., *Am. J. Physiol.*, 1924, lxx, 412.

[3] Hill, L., and Flack, M., *Fr. Physiol.*, 1908, 38, *Proc. Physiol. Soc.*, lvii.

The data presented in Fig. 1 show that there are 3 distinct changes in the respiratory movements when a subject is placed in a water bath varying from 65°F to 116°F. During the filling of the tub the movements are irregular, becoming slightly faster and much


FIG. 1.
The effect of submersion in water at various temperatures on respiration.

deeper while the bath remains at 65°F. As the temperature is increased, the respiratory movements tend to become more quiet and much shallower. At 80°F the movements are practically normal as to rate but are very shallow. The shallow characteristic begins to disappear at 110°F. When 115°F is reached, the respiratory movements again become deep and irregular, assuming a form somewhat similar to those recorded in the cold bath.

In most cases, very little discomfort was experienced in the cold bath. However, when the temperature reached 105°F, the subjects perspired profusely. As the temperature was increased still more, symptoms such as nausea, dizziness and extreme irritability were observed.

The outstanding feature of the experiment is that the respiration rate is changed but little, compensation being accomplished by a change of depth.

4664

## A Study of the Metabolism of Reticulocytes.

A. P. BARER, R. J. NEEDLES AND C. W. BALDRIDGE.
(Introduced by Fred M. Smith.)

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The metabolism of blood cells can be estimated from their glycolytic activity *in vitro*. This rate of glycolysis has been studied in the blood of patients with pernicious anemia during a remission induced by liver extract. In all of 5 cases the amount of sugar glycolyzed increased abruptly at the beginning of the remission. In 2 of the 5 cases the increased glycolysis during the reticulocyte crisis could be explained by an increase in erythrocytes and leucocytes. In the other 3 cases some of the increased glycolysis seemed to be attributable to the increase in the reticulocytes *per se*. This might indicate that young blood cells have a more active metabolism than adult cells.

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## An Experimental Study on the Nictitating Membrane of the Frog, Rana pipiens.

VERLUS F. LINDEMAN. (Introduced by J. H. Bodine.)

*From the Department of Zoology, State University of Iowa.*

The larval nictitating membrane, as it occurs in the frog tadpoles prior to the onset of metamorphosis, consists of a small mass of tissue imbedded within the integument bordering the anterior end of the eye socket. During metamorphosis this mass of tissue undergoes rapid growth which results in the formation of the nictitating membrane.

It seemed likely that extirpation and transplantation experiments with this mass of tissue before its transformation might throw some light upon the nature of the development of the nictitating membrane. By means of operative technique parts of it were removed and transplanted to other locations on the body. The animals used were normal tadpoles of the species, *Rana pipiens.*

The total extirpation of the integument in the region anterior to the eye socket in which this tissue is imbedded resulted in the failure of the nictitating membrane to be formed during metamorphosis. Removal of any part (*i. e.,* anterior half, posterior half, dorsal or ventral half) of this mass of tissue in the larvae, failed to hinder in any way the formation of a complete and well developed nictitating membrane during metamorphosis.

Autoplastic transplants to the back, of a section of the integument surrounding the eye and including the conjunctiva and the undifferentiated mass of tissue, resulted, during metamorphosis, in the formation of a mass of tissue resembling a partially formed nictitating membrane.

Extirpation of the integument along the ventral border of the eye which forms the lower eyelid and upon which the nictitating membrane normally forms, resulted in the partial regeneration of this area and the formation of a perfect nictitating membrane during metamorphosis.

It may be concluded that the small mass of tissue imbedded within the integument at the anterior border of the eye is undifferentiated in the larval stages of the frog, and that growth and differentiation are due primarily to hormonic influences released into the blood stream during metamorphosis.

# Illinois Branch.

*Chicago Medical Society, November 27, 1929.*

## 4666

## Effect of Therapeutic Venous Ligation on Blood Flow in Cases of Arterial Occlusion.*

M. LAURENCE MONTGOMERY. (Introduced by L. R. Dragstedt.)

*From the Department of Surgery of the University of Chicago.*

That the incidence of gangrene in a healthy extremity, whose main arterial supply has been suddenly interrupted, is reduced by the simultaneous or early obstruction of the concomitant or proximal venous return has been demonstrated repeatedly by clinical and experimental observers.

The physiological explanation of this phenomenon, however, is in part still the subject of controversy. It is recognized by all recent workers that ligation of the venous return causes an increase in the blood pressure of the affected extremity. Opinions as to its effect upon the flow of blood, however, differ widely. Brooks and Martin[1] believe that this procedure decreases the blood flow, while Holman and Edwards[2] maintain that the blood supply is increased. Neither of these 2 groups of workers measured the flow of blood directly.

Recently there has been devised in our laboratory a direct, continuous volume flow apparatus, a modification of the Ludwig stromuhr and of Stolnikow's double inlet-outlet apparatus, which is controlled by an electrically regulated valve.[3] By means of this instrument we have measured directly the changes in blood flow to an extremity, resulting from ligation of its principal peripheral arterial

* This work has been conducted under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

[1] Brooks, Barney, and Martin, K. A., *J. Am. Med. Assn.*, 1923, lxxx, 1678.

[2] Holman, Emilie, and Edwards, Muriel E., *J. Am. Med. Assn.*, 1927, lxxxviii, 909.

[3] Montgomery, M. L., and Lipscomb, T. H., *Am. J. Physiol.*, 1929, xc, 454.

supply and venous return. A preliminary report on this work forms the basis of this paper.

Male dogs, weighing 13 to 17 kg. were anesthetized by the intravenous injection of 200 mg. per kg. of sodium barbital. Coagulation was prevented by 30 mg. of heparin per kg. The volume flow apparatus was placed in the right iliac artery, thus measuring the blood which entered the right femoral artery as well as the chief collateral arterial supply to the right leg. The systemic blood pressure was taken from one of the carotid arteries. The right superficial femoral artery and the right common iliac vein were exposed to permit ligation.

A control record was taken of the blood flow through the iliac artery. Then the superficial femoral artery was ligated and a blood pressure cannula placed in its distal end. After the rate of blood flow then reached an apparent equilibrium, the right common iliac vein was occluded. This caused a sharp fall in the blood flow of from 10 to 15 cc. per minute (a drop of 20 to 30% from the preceding level), which was associated with the expected rise in the peripheral blood pressure of the right leg. To control this result, the occlusion of the common iliac vein was released and there followed a rapid rise in the blood flow of from 10 to 20 cc. per minute. This was accompanied by a fall in the peripheral blood pressure.

We have shown then, by direct measurement, that in a healthy extremity whose blood supply has been reduced by ligation of its main peripheral artery, ligation of its venous return proximal to the point of arterial ligation causes a sharp fall in the per minute flow of blood to the extremity.

## 4667

## Embryo-Arsenic Tumors in Rats.

F. A. MCJUNKIN AND M. F. CIKRIT.

*From the Department of Pathology, Loyola University School of Medicine.*

Carrell[1] by the injection of chick embryo pulp in conjunction with dilute arsenious acid obtained in fowls a tumor of sarcomatous type. The result was the same whether the embryo was mixed before injection with the arsenious acid or the two injected simultaneously at different places. White[2] repeated these experiments and confirmed

[1] Carrell, A., *Compt. rend. Soc. de Biol.*, 1925, xciii, 1083.

[2] White, A. W. M., *J. Cancer Res.*, 1927, xi, 111.

the results. Askanazy[3] transplanted rat embryo into grown rats and added Fowler's solution to their drinking water. In each of 2 series in which arsenic was used one rat was found to have a tumor of a malignant character. In one, the examination was made 13 and in the other 15 months after the embryo transplant. In the experiments of Carrell and of White the tumors developed as early as the ninth day and rather frequently in the fowls receiving the injections. From the results obtained in these investigations it appears that the response in the rat as compared with fowls is slower and less certain. The aim of our experiments was the investigation in rats of some of the variable factors, especially sites of injection and ages of embryos, which might explain slow and uncertain results.

*Experiment I.* The usual procedure with minor variations has been to draw much of the embryo into a syringe fitted with a short needle having 1 mm. lumen and at once to draw dilute arsenious acid to the 2 cc. mark. Of this suspension 0.5 to 1 cc. was injected. The time elapsing from removal of living embryo to injection was under 5 minutes. One of us (McJunkin) injected the testicles of 7 rats with a suspension of 3 mm. embryos in 1-150,000 arsenious acid. Six weeks later there was found in the testicle of one rat a minute opaque focus which microscopically consisted chiefly of cysts filled with cornified material. No trace of the embryo injection was found in the other testicles.

*Experiment II.* (a) 5 rats. Embryos measured 3 mm. and the arsenious acid was 1-1000. The suspension was injected into a ligated segment of one uterine horn. Forty days later the inner surface of the ligated uterus was smooth and contained none of the cells injected. (b) 5 rats. A duplicate of (a) except that embryos measured 10 mm. and the dilution of the arsenious acid was 1-25,000. After one month there was none of the transplanted embryo found either grossly or microscopically.

*Experiment III.* 11 rats. Embryos at term. Tissues tough and much of the aspirated part was liver, spleen and brain. Arsenious acid 1-25,000. Injection into the glutei muscles. Four months later the rats were killed and no evidence of the injected cells was found.

*Experiment IV.* 12 rats. Embryos 3 mm. Arsenious acid 1-50,000. 1 cc. of suspension injected into the right thigh with much of it passing into the loose intermuscular and subcutaneous tissue. The rats were killed after 122 days and tumors found in 2. Both were in the subcutaneous tissue over the muscles injected. The smaller (11x7x7 mm.) was a teratoma consisting chiefly of bone

[3] Askanazy, M., *Verhandl. d. Deutsch. Path. Gesellschaft*, 1926, 182.

and cartilage and presenting little evidence of active growth. The larger (18x8x7) was a soft mass in the groin attached to the muscles beneath. Histologically the growth consisted of a single type of tissue and was made up of large and small glands lined with a single to several layers of columnar epithelium which was actively proliferating. In many places several mitoses were found in single high lens fields. Some of the glands were considerably distended. The cystadenoma had invaded a small lymph node attached to the edge of the mass but no distant metastases were found.

*Experiment V.* 10 rats. Embryos 10 mm. long. Arsenious acid 1-50,000. Six of the rats also received arsenic in their drinking water. Autopsy in 108 days. Of the 4 rats not given arsenic in the drinking water 3 showed teratomata consisting of bone, cartilage and epidermal cysts situated either between the muscles or in the subcutaneous tissue. Of the 6 getting arsenic in the drinking water 2 showed teratomata: 1 small and the other 42x9x7 mm. The larger one in addition to bone and cartilage contained many cysts distended with cornified material, sebaceous glands and hair follicles.

The uterus is unfavorable for the growth of embryonic cells treated with arsenic. Apparently the arsenic-treated embryonic tissue grows more readily in the subcutaneous and intermuscular tissue than in testicle. The best results were obtained by injecting into the intermuscular tissue young embryos mixed with a 1-50,000 arsenious acid. Of the 50 rats in only one was there a tumor of single cell type with changes suggesting malignancy. It was a cystadenoma. The rôle of the arsenic in the production of these tumors was not determined.

In a very recent report Begg and Cramer[4] state that they have been unable to confirm the results obtained by Carrel and they are of the opinion that the experimental fowl tumors of Carrell, White and others may have been caused by a contamination of one or another of the materials inoculated with the virus of Rous sarcoma No. 1.

[4] Begg, A. M., and Cramer, A., *The Lancet*, 1929, ccxvii, 697.

4668

## Experimental Jejunal Ulcer.

G. B. FAULEY AND A. C. IVY.

*From the Department of Physiology and Pharmacology, Northwestern University Medical School.*

In the experimental study of the cause of jejunal ulcer following gastro-enterostomy, 3 factors have become quite evident as playing an etiological rôle; first, the irritating and digestive properties of the gastric contents; second, the motor drive of the stomach, and third, the possible greater susceptibility of the jejunal mucosa as compared to the duodenal mucosa to the first 2 factors.

We have devised an operation in the dog for the purpose of analysing the relative rôle played by these 3 factors. The operation consists in (1) dividing the duodenum about one inch below the pyloric sphincter and the jejunum about 12 inches below the ligament of Treitz; (2) then the distal end of the jejunum is anastomosed to the proximal end of the duodenum; (3) the distal end of the duodenum is closed and the proximal end of the jejunum is anastomosed (end-to-side) to the distal ileum about 15 inches from the ileo-cecal valve. The gastric chyme on being ejected strikes the first inch of the duodenum and then passes into the jejunum. There is no pancreatic juice or bile to play a rôle in neutralization, since these secretions are diverted to the lower ileum. The principle of the operation is that the acid factor is constant for both the duodenal

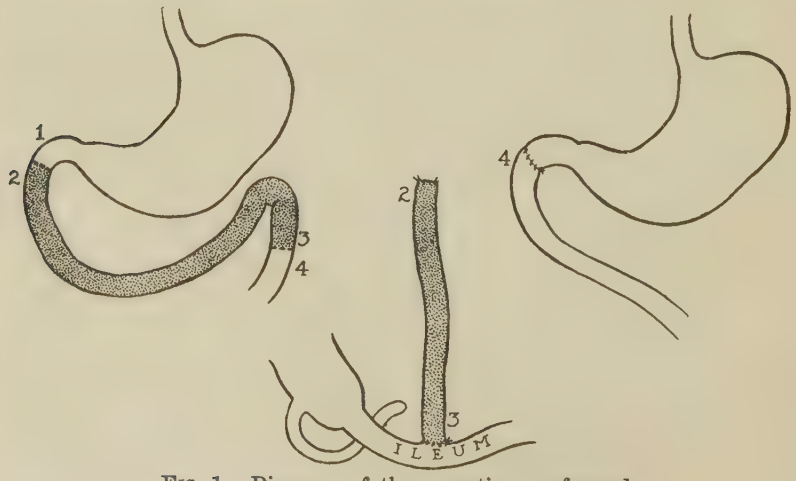


FIG. 1. Diagram of the operation performed.

and jejunal mucosa, and the motor drive of the stomach affects chiefly the duodenal mucosa. If an ulcer occurs only in the duodenal mucosa, it would mean that it was caused by the acid plus the motor drive factor. If an ulcer occurs in both the duodenal and jejunal mucosa, it would mean that the acid factor was the chief cause. If an ulcer occurs in the jejunal mucosa only, it would mean that its mucosa is more susceptible to the action of the gastric contents than the duodenal mucosa.

We have at the present time results on 4 dogs all of which died from a large perforating jejunal ulcer approximately 2 months after the operation. The dogs do very well for several weeks after the operation, after which they vomit intermittently, lose weight and eat only part of their food.

These results show quite decisively that the jejunal mucosa is more susceptible to the action of gastric contents than the duodenal mucosa.

## 4669

## On the Non-Existence of a Hormone for Salivary Secretion.

J. SACKS AND M. S. KIM. (Introduced by A. C. Ivy.)

*From the Department of Physiology and Pharmacology, Northwestern University Medical School.*

In view of the existence of both humoral and nervous mechanisms for the regulation of the secretory activity of the stomach and pancreas, it was decided to investigate the possibility of such dual control of the activity of the salivary glands. Although it has been tacitly assumed that no hormone mechanism for salivary secretion exists, the literature contains no definite statement on the point. Babkin[1] makes no mention of it in his monograph on the secretory activity of the digestive glands. Langley[2] and Malloizel[3] found that substances which caused a flow of saliva when applied to the tongue were without effect on the paralytic secretion by the submaxillary resulting from section of the chorda tympani nerve.

Two methods were used in this work. The mucous membrane of the mouth and tongue of several dogs was extracted with 0.4% HCl. The application of this reagent to the tongue normally produces a

[1] Babkin, B. P., *Die Äussere Sekretion der Verdauungsdrüsen*, Berlin, 1928.

[2] Langley, J. N., *J. Physiol.*, 1885, vi, 71.

[3] Malloizel, L., *J. de physiol. et de path. gen.*, 1902, iv, 651.

marked flow of saliva. The extract was injected intravenously into dogs under barbital-ether anesthesia in which Wharton's duct had been cannulated. Quantities of extract sufficient to lower the blood pressure from 60 to 80 mm. of Hg were injected without any evidence of increased flow from the cannula. In each case stimulation of the chorda tympani nerve gave a marked response.

The second method was a study of the effect of the application of 0.4% HCl to the tongue of a dog in which one submaxillary gland had been denervated. A fistula of Wharton's duct was prepared in each of 2 dogs. After recovery from the operation, the dogs were placed in the stocks daily, and 0.4% HCl was applied to the tongue. A copious flow of saliva resulted. Three weeks after the first operation, the chorda tympani and cervical sympathetic nerves were sectioned, and the daily applications of acid to the tongue resumed on the second day after the operation. The nerve section caused an immediate cessation of the flow of saliva from the fistula, and in no case was there any effect produced by the acid.

Although the results reported were obtained in only 4 acute experiments and 2 fistula dogs, they are so consistently negative that we feel them adequate to support the conclusion that there is no hormone mechanism for salivary secretion in the dog.

## 4670

### Attempts to Visualize the Gall-Bladder of the Rabbit with Tetraiodophenolphthalein.

H. C. LUETH AND A. C. IVY.

*From the Department of Physiology and Pharmacology, Northwestern University Medical School.*

In previous work[1] we were unsuccessful in attempts to record a gall-bladder contraction in the rabbit on the injection of cholecystokinin. It was reasonable to suppose by analogy from Graham's and Cole's[2] work, that we would be able to study the problem roentgenologically, after the administration of sodium tetraiodophenolphthalein. Graham, Cole and Copher[3] report that they have visualized the gall-bladders of rabbits after the subcutaneous injection of

[1] Lueth, H. C., Ivy, A. C., and Kloster, G., *Am. J. Physiol.*, 1929-30, xci, 329.

[2] Graham, E. A., Cole, W. H., Copher, G. H., *J. Am. Med. Assn.*, 1925, lxxxiv, 15.

[3] Copher, G. H., *J. Am. Med. Assn.*, 1925, lxxxiv, 1563.

sodium tetrabromphenolphthalein, but add that these shadows were faint and inconsistent.

We hoped to get more consistent results using intravenous injections of tetraiodophenolphthalein. Because of our early failure in 4 rabbits to get shadows with the usual doses of 0.2 gm. per kilo, we increased the amount. Four rabbits were given 0.3 gm. per kilo, one of which showed toxic symptoms. One of 2 rabbits given 0.4 gm. died in 4 hours. Twenty-four rabbits were given the usual dose of 0.2 gm. per kilo, which proved fatal in 2 cases. This is in accord with Whitaker and Milliken,[4] who studied the toxicity of tetraiodophenolphthalein and tetrabromphenolphthalein, finding that 0.24 gm. is the largest single non-toxic dose for rabbits. Because of the possibility of missing the time of maximum concentration, which in dog and man is after 14 hours, the dye was given to fasting animals, and pictures taken every 2 hours until 40 hours after the injections. We were uniformly unable to obtain shadows of the rabbit's gall-bladder. As control experiments we injected the same dye, in the same dose (0.2 gm. per kilo) in dogs and sharply defined shadows were obtained.

Because of this lack of concentration of the dye in the rabbit, we took specific gravity determinations. We found the rabbit's gall-bladder bile to have a specific gravity of approximately 1.048, which is within the lower range of the specific gravity of gall-bladder bile of man and dog.

In one experiment in which the animal was killed by the injection of 0.4 gm. per kilo no shadow of the gall-bladder was discernible. On the death of the animal, 10 minutes later, a picture of the excised liver and gall-bladder was made. In this picture the gall-bladder was visualized but its shadow was no more dense than the more dense portions of the liver. This may be the reason why the rabbit's gall-bladder does not visualize, since it is so completely surrounded by the lobes of the liver.

[4] Whitaker, L. R., and Milliken, G., *Surg. Gynec. Obst.*, 1925, xl, 17.

4671

## On the Non-Ubiquitous Occurrence of Secretin.

G. E. DREWYER AND A. C. IVY.

*From the Department of Physiology and Pharmacology, Northwestern University Medical School.*

After Bayliss and Starling found that extracts of the upper intestinal mucosa on intravenous injection would cause the pancreas to secrete, numerous investigators have reported that such an active principle was present in various animal and plant tissues. Such an active principle has been reported to be present in Witte's peptone, stomach and colon mucosa, muscle tissue, brain, thyroid, liver, parathyroids, pineal gland, mammary gland, spinach, nettle, and hydrolized egg-white. All these extracts contain a blood pressure reducing substance; and since histamine increases pancreatic secretion, it may be that the augmentation of pancreatic secretion caused by these various tissue extracts may be due to vaso-dilation and not to a specific substance or secretin.

Having at hand the method of Weaver, Luckhardt and Koch[1] which yields secretin from the upper intestinal mucosa of dogs, hogs, sheep, cattle and man free of vaso-depressor substances, we decided to apply this method to various body tissues and to spinach. The tissues were collected from freshly killed animals, and were cut into small pieces, and 0.4% HCl was added in the proportion of 2000 cc. per kilo of tissue. The acid was allowed to remain in contact with the tissue for ½ to 1 hour, with frequent stirring. The acid extract was then strained off through gauze, and immediately saturated with NaCl (30 gm. per 100 cc.), as soon as the salt was dissolved the precipitate was filtered off, the filtrate being called B. The precipitate was then taken up in water and the insoluble residue filtered off and discarded. The soluble portion was called A. The extract A was used for injection. The extracts from the various tissues, brain, bone, liver, lung, kidney, pancreas, thyroid, stomach, intestine, colon, muscle, heart and spinach were injected in doses varying from 5 to 50 cc. No increase in the secretion of the pancreas was noted with any of these tissues except the extract of intestine and stomach. All extracts were vaso-dilatin free. Controls were run on the pancreas of each dog to see if the pancreas was active to secretin stimulation, by injections of a known solution of

[1] Weaver, M. M., Luckhardt, A. B., and Koch, F. C., *J. Am. Med. Assn.*, 1926, lxxxvii, 640.

secretin. This procedure was again repeated at the termination of the experiment to see if there was any variation to the original dose. Two sets of extracts were made and 6 dogs were run, with similar results in every case. Further, if we injected the salt filtrate B into the animal, there occurred a fall in blood pressure, varying according to the dose and the tissue used, from 25 to 100 mm. of Hg. In all such cases the pancreas was slightly stimulated, the amount of secretion produced depending on the degree of fall in blood pressure and its duration.

These results show that the secretion caused by various tissue extracts other than gastric and intestinal mucosa is due to the presence of vaso-depressor substances and not to the specific substance called secretin. About 10 times as much secretin can be extracted from the intestinal mucosa of the first 6 feet of the intestine as from the mucosa of the pyloric antrum. Before an extract can be said to contain secretin, it must be at least shown that it is free of vaso-depressor action.

4672

## The Intravenous Administration of Irradiated Ergosterol.*

C. I. REED AND E. A. THACKER.

*From the Department of Physiology, University of Illinois College of Medicine, Chicago.*

This investigation was begun in the hope that the intravenous administration of ergosterol might throw some light on the mechanism of its action, if it could be shown to be effective by this method. Normal dogs of 10 to 12 kilos weight were selected and confined to a stock diet of ground beef heart and Puppy Meal, 3:1. Ten animals have now been studied. Routine determinations of calcium and inorganic phosphorus were made. Figure 1 shows the results on one animal, which is fairly typical of the series. After a short period of preliminary observation, injections were begun of ergosterol in corn oil. This oil alone, in amounts up to 15 cc. does not produce any of the observed effects. In the illustrative case. approximately 20 mg. were administered daily during 2 weeks. The

* Supplied by Standard Products Co., formerly The Fleischmann Co. This investigation was supported in part by a grant from the Phi Rho Sigma Medical Fraternity, and from the Phi Sigma Biological Society.

calcium concentration was increased, but there were pronounced fluctuations for which no explanation can be offered at present. During 10 days, the animal gained weight rapidly and became extraordinarily active. During this time also, there were pronounced fluctuations in the concentration of inorganic phosphorus, but the average level was unchanged. At the end of this period it became apparent that the calcium concentration was decreased almost to the original level; so the daily dose was increased to 30 mg. The result was a second increase in calcium to almost 16 mg. at the end of another week. The dose was now increased to 50 mg. On the 25th day after the first administration, the calcium concentration was increased to almost 19 mg.; at this time the phosphorus had fallen to 4.7 mg. The dog had now begun to lose weight and had become less active. At the termination of the observation, post mortem examination of various organs, particularly of the parathyroids, will be made.

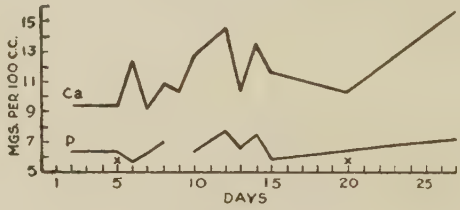


FIG. 1.

In the case of 2 other animals which received the maximum dose at an earlier stage, tetanoid spasms have been observed, which resembled superficially the spasms resulting from an overdose of parathyrin, but neither calcium nor phosphorus concentrations were excessively high at this time. These dogs have lost weight much more rapidly. Neither one ever showed any increased activity.

Other blood changes are being studied. Also, a study of urinary excretion and of metabolism will be undertaken.

4673

## Effect of Certain Alkaloids Upon Early Cleavage in Arbacia Punctulata.

MARIE A. HINRICHS AND IDA T. GENTHER.

*From the Department of Physiology, University of Chicago, and Washington University, St. Louis.*

In order to study the physiological effects of certain alkaloids upon some of the lower organisms, and to determine, if possible, whether there was any evidence of specificity of action in these forms, the following experiments were planned: (a) A study of the effects of caffein on some of the physiological and morphological processes of the young and adult forms of *Planaria dorotocephala*, an organism with relatively labile tissue; (b) a study of the effects of caffein, atropine, and pilocarpine on cleavage and on the embryonic development of one of the lower organisms in which differentiation is rapid enough to permit of modification under the influence of agents acting only for a short period of time.

The first series of experiments, (a), concerned themselves with a study of the effects of caffein upon oxygen consumption, carbon dioxide production, and head frequency in *Planaria dorotocephala*. A record of differential disintegration in high concentrations of caffein also indicated a differential susceptibility to this alkaloid. No specificity of action was found for caffein in this form, and both stimulation and inhibition were recorded in the studies made with head frequency and with respiration, the effect depending upon the concentration used, and the length of the period of exposure.[1]

The experiments on early cleavage will be reported in this paper, and studies on differential modification of development in *Arbacia punctulata* will be reported in the following paper. In the study of the effects of various concentrations of alkaloids on the rate of early cleavage of *Arbacia* eggs, the following procedure was observed: Stock solutions of a 0.5% concentration were made up for each alkaloid. From these, further dilutions were made up such that concentrations ranged from 0.1 cc. to 10 cc. of stock solution per 100 cc. of sea water. The eggs of several females were mixed, fertilized, and washed, and then measured quantities of a uniform suspension of such eggs were placed in a series of varying concentrations of alkaloids, and studied at intervals to find out whether the rates of early cleavages could be modified as compared to the rate of cleav-

[1] Hinrichs, M. A., *J. Exp. Zool.*, 1924, xl.

age of a control lot of eggs kept in sea water. After such preliminary studies were made, the eggs were transferred to Erlenmyer flasks, and more alkaloid solution added. The original concentration was maintained.

*Results:* Counts recorded cleavage in 50% or more of each lot of eggs. It was found that with caffein, the first cleavage and second cleavage appeared at the same time as in the control in all dilutions up to 5 and 10 cc. per 100. (.025%-.050% concentration.) In these higher concentrations, cleavage was delayed to nearly twice the normal length of time in the first division, to one and a third times the normal period in the second division, and only slightly, in the third division. In Pilocarpine solutions, there was no apparent delay during the first cleavage. (In dilutions of Pilocarpine hydrochloride, there was even a suggestion of a slight stimulation in rate in concentrations as low as 0.2 to 0.6 cc. per 100. (.01-.06%) The nitrate solution was more strongly inhibitory than the hydrochloride of Pilocarpine, although there was no delay in the first cleavage. The second division in the nitrate series was delayed by concentrations as low as 0.3 cc. per 100, the time of cleavage appearing about 30 minutes later than the controls. The delay in cleavage rate was maintained in the Pilocarpine solutions through the fourth cleavage. Atropine showed by far the most rapid inhibitory effect on cleavage rate, *viz.*, 0.2 cc. per 100 (.01%), in the first cleavage. In the second and third cleavages, the delay did not appear until concentrations as high as 1 cc. per 100 were reached. In the fourth cleavage, concentrations up to 2 cc. per 100 showed no delaying effect. A slight stimulation in rate was recorded for the first cleavage in the 0.1 cc. per 100 concentration.

From the above data, it is evident that the effect on early cleavage is not marked until higher concentrations are reached with all alkaloids studied with the exception of Atropine. Also, the effect appears to "wear off" in later cleavages, again with the exception of higher concentrations of Atropine.

In order to study the effect of these various concentrations of alkaloids upon the rate of formation of top-swimming larvae, the eggs were now transferred to Erlenmyer flasks, and the time noted at which a ring of larvae formed at the top of the liquid. Fourteen series were studied with Pilocarpine nitrate, 7 series with Pilocarpine hydrochloride, 17 series with Atropine sulphate, and 6 with Caffein. Of the total number of 66 observations made with Pilocarpine nitrate, only 14% showed stimulation in speed of formation of top-swimming larvae, as compared to the seawater control.

All these were found in concentrations of from 0.4-3 cc. per 100, and from 7-9 hours after fertilization. 56% showed an inhibition in rate, and 30%, no effect. Forty-one observations were made with Pilocarpine hydrochloride, and of these, only 11% showed stimulation, 51% depression, and 38% no effect. This would seem to indicate that, in the concentrations used in these experiments, Pilocarpine is only slightly accelerative in its action on the rate of growth and division in this form.

Seventy observations were made with Atropine sulphate, and of these none were stimulative in rate of formation of top-swimming larvae, as compared to the control. Fifty-nine per cent showed inhibitory effects, and the rest produced no effect on rate of growth up to this stage. All inhibitory effects appeared within 7-9 hours after fertilization, after which time the effect seemed to wear off.

Of the 18 observations with Caffein, only 11% were inhibitory, none stimulative, and 89% showed no effect in the concentrations used.

From the above 2 types of experiment, it appears that Pilocarpine may be either stimulative or inhibitory, depending on the concentrations used. Caffein was practically without effect, except in the highest concentrations, which were inhibitory. Atropine is more strongly inhibitory than either Pilocarpine or Caffein, but may produce a slightly stimulative effect which wears off readily.

By the time 12 hours have elapsed since fertilization, the effect of all 3 alkaloids seems to be negligible, except in higher concentrations. In order to test the further effect on development of these embryos, studies were made, using the same concentrations, and developmental rates of various body regions were observed over longer periods of time. The results of such studies will be briefly summarized in the following paper.

4674

## Modification of Development in Arbacia Punctulata on the Basis of Differential Susceptibility to Certain Alkaloids.

MARIE A. HINRICHS.

*From the Department of Physiology, The University of Chicago.*

In order further to test the effects of the alkaloids, Pilocarpine, Atropine, and Caffein upon development of *Arbacia* embryos, the following experiments were made: Fertilized eggs were allowed to develop in the various concentrations of alkaloids, as in previous experiments reported in the preceding paper, and in a paper by Mathews.[1] A 0.5% solution was used as a stock solution, from which dilutions were made from 0.1 to 10 cc. per 100 cc. of sea water.

The eggs were allowed to develop for 48 hours, after which time micrometer measurements were made of arm length, body length, arm width across the tips, width of the base, and the base and hypotenuse of a triangle which would include the oral lobe in optical section. Twenty-five embryos were measured from eggs of each of 3 females, for each concentration studied, and compared with the controls, measurement for measurement. On the basis of these calculations, it was possible to obtain percentage values for each dimension which indicated increase or decrease in growth rate relative to the same dimension in the control.

It was found that there may be either stimulation or inhibition of development in *Arbacia larvae,* and that the effect is dependent on the concentration of alkaloid used. As previously observed, Atropine is more strongly inhibitory than either Pilocarpine or Caffein, and Pilocarpine is more strongly stimulative than either Atropine or Caffein. Also, it was obvious that the effect in any case was differential. The regions of most rapid growth, *i. e.,* the oral lobe region, and the aboral arms and median region between them, are most rapidly and most completely modified in their rates of growth. Both differential stimulation and inhibition were obtained with each alkaloid, depending on the concentration used.

[1] Mathews, *Am. J. Physiol.,* 1901, vi.

# New York Meeting.

*New York Academy of Medicine, December 18, 1929.*

## 4675

### Utilization of Fat by Resting and Exercising Muscles of Diabetic Dogs.

H. E. HIMWICH, H. FRIEDMAN, E. BERRY AND W. H. CHAMBERS.

*From the Yale University College of Medicine, New Haven, Conn.*

Eight normal, 13 phlorizinized, and 9 depancreatized dogs under amytal anesthesia were studied during rest and exercise. Blood entering and leaving muscle and liver was analyzed for fat, using the method of Stewart and White.[1] The experimental error was ±20 mg. %, and differences of 40 mg. % or more were considered significant. The differences of the arterial and venous fat contents of the blood of the muscles of the normal post-absorptive dogs varied, since the muscles liberated fat 5 times and removed it on 3 occasions. Of greater interest are the observations of the diabetic dogs, since in 10 of 13 significant determinations on the phlorizinized dogs, and 12 or 15 on the depancreatized animals, the muscles of the lower extremities removed fat from the blood passing through them.

The results obtained on the blood of the liver were not the same in the phlorizinized and depancreatized dogs. The livers of the phlorizinized dogs usually removed fat from the blood, while those of the depancreatized dogs added fat.

TABLE I.

*Typical results of the fat content of the blood of diabetic dogs.*

| Date | No. | Femoral Artery | Femoral Vein | Portal Vein | Hepatic Vein | Remarks |
|---|---|---|---|---|---|---|
| 7/10/27 | 1 | 1345 | 1198 | 1310 | 1379 | Depancreatized Exercise |
| 7/10/27 | 2 | 1408 | 1221 | 1337 | 1488 | Depancreatized Exercise |
| 7/19/27 | 3 | 1156 | 1039 | 1017 | 1251 | Depancreatized Rest |
| 3/21/27 | 4 | 825 | 654 | 547 | 529 | Phlorizinized Rest |
| 5/17/27 | 5 | 574 | 404 | 538 | 466 | Phlorizinized Rest |
| 5/23/27 | 6 | 879 | 789 | 618 | 592 | Phlorizinized Rest |

[1] Stewart, C. P., White, A. C., *Biochem. J.*, 1925, xix, 840.

Thus these results obtained by the method of Stewart and White[1] indicate the utilization of the fat of the blood by the muscles of diabetic dogs.

## 4676

## The So-called Hyperglycemic Action of Insulin.

I. NEUWIRTH, F. CO TUI AND G. B. WALLACE.

*From the Department of Pharmacology, New York University.*

Collens and Murlin[1] have recently reported that the portal injection of insulin into dogs, in dosage of 0.05 to 0.1 unit per kilo weight, results in an immediate sharp rise of blood sugar of 20 to 80 mg. The rise occurs within 5 minutes and is then followed by a rapid decline. No such rise occurs following the systemic injection of the same dose of insulin. Bürger and Kramer[2] at about the same time reported that the injection of 10 to 20 units of insulin into the cubital vein of human beings produces a rise of blood sugar averaging 11.5%; intrajugular injection of 40 units into dogs of about 20 kilograms causes a rise averaging 28%; intraportal injection results in a rise averaging 46%. The rises occur within 5 minutes and are followed in 10 to 30 minutes by a rapid fall. In both of these reports, the results are interpreted as showing that insulin has a glycogenolytic action on the liver and that the hyperglycemia is a physiological or normal response to this action. Since such an action of insulin would have a bearing on work we were carrying out, we have gone into the matter in order to have a clearer understanding of its significance.

We have carried out experiments on dogs, corresponding to those described in the reports cited. We employed the Lilly insulin, as did Collens and Murlin, whereas the Burroughs Wellcome product was used by Bürger and Kramer. Using small doses, 0.1 unit per kilo, we obtained no rise in blood sugar on intrajugular injection, but a rise followed intraportal injection. Our maximum rise, however, was 15 mg. as compared to the 20 to 80 mg. rise of Collens and Murlin. With the larger dosage, 2 to 3 units per kilo (40 units total), we obtained a rise on intrajugular injection of 5 to 10%, and

[1] Collens, W. S., and Murlin, J. R., PROC. SOC. EXP. BIOL. AND MED., 1929 xxxvi, 485.

[2] Bürger, M., and Kramer, H., *Ztschr. f. d. ges. exp. Med.*, 1929, lxv, 487.

on intraportal injection of 15%, as compared to the 28% and 46% averages of Bürger and Kramer. Our results, however, are confirmatory of the ones reported in regard to the occurrence of a rise.

Through the kindness of Doctor Geiling we were able to secure some crystalline insulin prepared in the Johns Hopkins laboratory, and we have repeated our experiments, using instead of the Lilly insulin, the crystalline product in corresponding dosages.

When this crystalline insulin is injected intrajugularly, or intraportally in dosages of 0.1 unit or 2 to 3.5 units per kilo a fall of blood sugar comes on within 6 to 10 minutes with no rise at 3 to 6 minute intervals after the injection. Since the only difference in procedure here is in the form in which the insulin is administered, it appears that the hyperglycemia when obtained, is not a true insulin action, but is due to some substance in the commercial products, which acts particularly on the liver. Fisher's[3] experiments in which he showed that there could be obtained from the pancreas and other tissues, a toxic substance which among other actions, caused a rise in blood sugar, are of interest in this connection.

## 4677

## Influence of Acid and Base-Forming Feeding on Growth.

EMMA LOUISE SAMUEL AND I. NEWTON KUGELMASS.

*From the Department of Pediatric Research, The Fifth Avenue Hospital, New York.*

Lack of accord between infant feeding practice and metabolic principles has led to the present investigation. The effects of natural acid- and base-forming diets on the growth and metabolism of young rats have been studied from the physical, chemical and pathological standpoints.

Comparative studies were made on 2 groups of 22 and 25 young albino rats weighing between 30 and 35 gm. Litters of the same stock and age were maintained on acid- and base-forming dietaries, in sunlight and in darkness, during all the seasons.

At the end of 28 weeks the dietary acidity and alkalinity showed marked effect on the animals. The acid-forming diet depressed on the average the serum phosphorus from 6.1 to 5.7, the alkaline reserve from 48 to 44 vol. per cent, and total base from 148 to 137

[3] Fisher, N. F., *Am. J. Physiol.*, 1923, lxvii, 57.

with a compensatory rise in the chloride from 280 to 305 and cholesterol from 140 to 150. The base-forming diet produced the converse changes, serum phosphorus from 6.1 to 7.5, alkaline reserve from 48 to 56 vol. per cent, total base from 148 to 154 vol. per cent. The albumin-globulin ratios, percentage fat and iodine numbers of the fat were all normal. The hemotological data were also unaltered in both groups.

The animals maintained on acid-forming diets showed rickets clinically and histologically while those fed on base-forming diets showed normal bones. The animals on the base-forming diet in particular showed the greater percentage of ash, 61%, and had heavier bones while those on the acid-forming diet showed 54% of ash according to analyses of the femora. The animals that had been exposed to the light showed a higher ash content, 54% compared with 49% of those in darkness. The rats on base-forming diets showed a more marked gain in weight, 17 gm. per week as compared with 7 gm. per week for the animals on the acid-forming diets. This weight contrast was striking for the summer, less so for the spring and least for the winter.

4678

## Effect of Division of Dorsal Roots of Cervical Nerves Upon Diaphragmatic Respiratory Movements.

HELEN C. COOMBS.

*From the Department of Physiology, New York Homeopathic Medical College and Flower Hospital.*

In a study of the neuro-muscular mechanism of respiration, carried out on cats, it has been observed that, in many cases, division of the vagi is followed not only by a marked decrease of the respiratory rate, but also by a diminution in amplitude of diaphragmatic movements, while costal movements increase in depth. Subsequent section of the dorsal cervical (phrenic) nerve roots in these cats is attended by very little further change in respiratory rate or movement.

When, on reversing the order of experimental procedure, the dorsal roots of the cervical nerves (iii, iv, v, vi) are divided first, both a diminution in amplitude of diaphragmatic contractions and slowing in rate ensues, and subsequent division of the vagi brings about almost no further change in respiratory rate.

That respiratory impulses to the diaphragm may still bring about increased depth of diaphragmatic movement after section of the dorsal roots of the cervicals, has been shown by division of the dorsal spinal nerve roots of the intercostals in the thoracic region with the resulting diminution in costal movement. Under such conditions, there is an increase in the magnitude of diaphragmatic movement. This is similar to the increase in costal movements observed by us some years ago when, after division of the dorsal spinal nerve roots in the thoracic region, the phrenics were excised and an increase in the magnitude of costal movement was observed.[1]

The fact that diaphragmatic movement and not costal, is diminished in magnitude by division of the vagi, appears to indicate a closer relationship of the vagi to the phrenics than to the thoracic roots of the nerves concerned in the control of costal respiratory movements. These latter appear to have central stations as high as the posterior *corpora quadrigemina,* section behind which affects costal respiratory movement to a much greater extent than diaphragmatic.[2]

In our earlier work,[3] the decrease both in magnitude and rate of costal movements on division of the thoracic and cervical dorsal spinal nerve roots was pointed out. It is now suggested that a distinction may be made between the decrease in *amplitude* with little change in rate, produced by division of the dorsal roots of the spinal nerves in the thoracic region, and the decrease in *rate* which seems to be a more characteristic result of the division of the dorsal roots of the spinal nerves in the cervical region, particularly those of the phrenic nerves from the diaphragm.

[1] Pike, F. H., and Coombs, H. G., *Science,* 1922, lvi, 691, and the papers there cited.

[2] Coombs, H. C., *Science,* 1929, lxx, in press.

[3] Coombs, H. C., *Am. J. Physiol.,* 1918, xlvi, 459.

4679

## A Method for Determining the Chill-Producing Properties of Anti-Pneumococcic Serum.*

A. B. SABIN AND G. B. WALLACE.

*From the Departments of Pharmacology and Bacteriology, University and Bellevue Hospital Medical College.*

Following the intravenous injection of concentrated, anti-pneumococcus horse serum, there frequently occurs a systemic reaction of varying intensity. The reaction comes on usually in from 30 to 60 minutes after the injection, and begins almost always with a chill, which is accompanied and followed by an elevation of temperature.

It seems that this reaction is not an essential factor in the beneficial effects of the serum, indeed it is considered by many to be a distinct disadvantage and to be responsible for some limitation of a more general employment of the serum therapy. Unfortunately there has been no method devised to determine, in advance of its use, whether the serum to be employed contains chill-producing properties, and so this knowledge is obtained only after the occurrence of the reaction in a treated patient. The present communication is a report of a successful imitation of the reaction in dogs, thus affording a method of determining before its clinical employment, the presence or absence of the chill-producing factor in any given sample of serum.

In the first experiments, it was found that a serum which had produced a typical reaction in a patient, produced a corresponding reaction when injected into the jugular vein of a dog. On the other hand, a serum which had failed to give a reaction in a patient, also failed to give one in a dog. Following these initial experiments, a considerable number of serums, which had been or subsequently were used in the pneumonia wards at Harlem Hospital, were tested. The experimental results were in striking agreement with the clinical ones.

The procedure in the animal test is as follows: The dogs selected are of the short-haired variety, of a weight between 5 and 10 kilos; the temperature is taken by rectum before and at 20 minute intervals after the serum injection; the thermometer is inserted the same distance for each reading; the serum is injected into the jugular vein, no local anesthesia or operative exposure being necessary.

* Aided by a grant from the Littauer Fund for the Study of Pneumonia, New York University.

The criterion of a positive reaction is a rise of temperature of 1.5°F or more, occurring within 60 to 75 minutes, and maintained for at least an hour. A chill may or may not be present, apparently unrelated to the degree of temperature elevation. In a typical, strong reaction, there is first a definite chill, coming on within half an hour after the injection; the rise in temperature is observed about 10 minutes later, and reaches a maximum of 2°F or more in about 15 minutes. The temperature remains elevated for 2 hours or longer The dose of the serum required to produce effects varies from 1 to 10 cc., practically the same as that required in patients.

Just as in patients, there is some variability in the responsiveness of different dogs to the test, and it is desirable in the selection of animals to make a preliminary control test, not only to determine the reaction to a known chill-producing serum, but the lack of reaction to a non chill-producing serum as well. The same dog may be used 6 or more times without the development of tolerance. On the other hand if the injections are too widely spaced, anaphylactic phenomena occur.

The accompanying table is illustrative of the experimental results.

TABLE I.

| Serum | Test on Dogs | | Test on Patients | |
|---|---|---|---|---|
| | Dose | Effect | Dose | Effect |
| | cc. | | cc. | |
| No. 1 | 1 | +2.2°F | 2 | Chill |
| " 1 | 5 | Marked chill, +3.5°F | — | — |
| " 2 | 10 | None | 10 | No chill |
| " 3 | 4 | Marked chill, +3.3°F | 4 | Marked chill |
| " 7 | 10 | None | 20 | No chill |
| " 11 | 10 | +1.0°F | 10 | Mild chill |
| " 14 | 5 | +1.9°F | 5 | Chill |
| " 14 | 10 | Chill, +2.2°F | — | — |
| " 18 | 10 | +2.0°F | 10 | Chill |
| " 23 | 3 | +1.2°F | — | — |
| " 23 | 5 | Chill, +2.0°F | 5 | Chill |
| " 24 | 10 | None | 10 | No chill |

From work already done it appears possible to differentiate chill-producing from non chill-producing serums before these have been subjected to the various procedures for refinement. This, and attempts to determine the nature of the chill-producing factor will be reported later.

4680

## Calcification of Teeth and Bones on Rachitic and Non-Rachitic Diets.*

MAXWELL KARSHAN. (Introduced by William J. Gies.)

*From the Laboratory of Biological Chemistry, the College of Physicians and Surgeons, and the School of Dental and Oral Surgery, Columbia University.*

Changes in teeth as a result of deficient diets have been reported by a number of investigators. McCollum, Simmonds and Kinney[1] produced a number of defects in the teeth of rats by feeding rachitic diets. Orban,[2] by means of histological examination, found poor calcification in the incisor teeth of rats on a number of deficient diets. Mellanby[3, 4] reported poor calcification in the teeth of dogs and rabbits on rachitic diets. Toverud[5] obtained a small reduction in the ash, calcium, and phosphorus in the teeth of rats on a diet deficient in calcium. Perlzweig[6] found decreases in calcium and phosphorus in the incisor teeth of rats on diets low in either calcium or phosphorus, the low calcium diet giving a greater decrease than the low phosphorus diet.

The object of this investigation was to determine whether the calcium, phosphorus, and ash of the incisor teeth of rats would undergo changes similar to those produced in bone by a rachitic diet known to produce marked changes in bone. The Steenbock diet as modified by Epstein[7] was employed. This consists of the following and will be referred to as the basal diet:

Yellow corn meal 66%, wheat gluten 20%, egg albumin 10%, calcium carbonate 3%, sodium chloride 1%, spinach 10 gm. per rat per day, water *ad lib.*

Excluding the spinach, this diet contains 1.22 gm. Ca and 0.09 gm. P. (ratio 13.5:1) in 100 gm. of the ration and produces marked rickets accompanied by good growth.[7] The addition of 2% $KH_2PO_4$ or 2% cod liver oil to this diet prevents rickets.[7]

* This work has been conducted with the aid of a grant from the Chemical Foundation for research in biochemistry.

[1] McCollum, Simmonds, Kinney and Grieves, *Johns Hopkins Hosp. Bull.*, 1922, xxxiii, 202.

[2] Orban, B., *J. Am. Dental Assn.*, 1927, xiv, 1619.

[3] Mellanby, M., *Biochem.* J., 1926, xx, 902.

[4] Mellanby, M., *British Dent. J.*, 1923, xliv, 1031.

[5] Toverud, G., *J. Biol. Chem.*, 1923, lviii, 583.

[6] Perlzweig, W. A., *J. Allied Dental Soc.*, 1916, xi, 70.

[7] Epstein, N., Dissertation, Columbia University, 1928.

The teeth and bones (femur) were compared on 2 groups of diets as follows:

Group I—(1) Basal; (2) Basal + 2% $KH_2PO_4$; (3) Basal + 2% $KH_2PO_4$ + 2% cod liver oil.

Group II—(2) as in group I; (3) as in group I; (4) Basal supplemented after 30 to 45 days by 2% $KH_2PO_4$; (5) Basal supplemented after 30 to 45 days by 2% cod liver oil.

After 30 to 45 days the rats in group I were killed with chloroform, and the incisor teeth and femurs were removed. The pulp and marrow (after splitting the bone) were removed by means of a dental pulp extractor. In several series the pulp and marrow were not removed. Rats in group II were killed after 64 to 80 days. In order to equalize conditions the rats in each comparative group were of the same litter and sex. There were at least 6 rats on each diet; 12 comparisons were made between the basal (rachitic) diet and the basal diet + $KH_2PO_4$. Analyses were made on the alcohol-ether extracted material by 2 methods: (1) the method of Shear and Kramer[8] in which the calcium and phosphate are removed from the tooth and bone powder by digestion with 1*N* HCl for 10 minutes (three to four times the weight of the sample specified in the method was employed); (2) after ashing.

We have been able to produce only slight variations in calcium, phosphorus, or ash of the teeth on any of the diets. Even when the bone calcium on the basal diet was reduced to 46% and the bone phosphorus to 38% of that on the basal diet + $KH_2PO_4$ + cod liver oil, the tooth calcium was practically unchanged and the phosphorus was reduced to the extent of only 4% of the total. In most experiments even this slight variation was not obtained.

The averages and average deviations of calcium and phosphorus of the teeth and bones on diets (1), (2) and (3) are given in the table. The figures represent the percentage of the dry weight of the alcohol-ether extracted material.

TABLE I.

| Diet | Teeth | | Bone | |
|---|---|---|---|---|
| | % Ca | % P | % Ca | % P |
| 1 Rachitic | 26.7 ± 0.6 | 13.8 ± 0.5 | 11.3 ± 1.2 | 5.9 ± 0.8 |
| 2 Rachitic + $KH_2PO_4$ | 26.2 ± 0.8 | 13.8 ± 0.6 | 20.3 ± 0.7 | 9.7 ± 0.4 |
| 3 Rachitic + $KH_2PO_4$ + cod liver oil | 26.5 ± 0.5 | 14.0 ± 0.1 | 21.8 ± 0.7 | 10.5 ± 0.5 |

[8] Shear and Kramer, *J. Biol. Chem.*, 1928, lxxix, 105.

Tooth growth, as measured by the weight of the freshly extracted tooth was as good on the rachitic as on the non-rachitic diets and varied approximately with the variations in the growth of the rats. The percentage of bone calcium, phosphorus and ash on the basal diet $+KH_2PO_4$ + cod liver oil was about 8% higher than that on basal diet + $KH_2PO_4$.

In the recovery experiments when $KH_2PO_4$ or cod liver oil was added after the rats had been on the rachitic diet for 30-45 days, the calcium and phosphorus of the bones were increased to about the same level in each case, but fell somewhat short of the calcium and phosphorus of the bones of rats that were on the basal diet + $KH_2PO_4$ from the outset of the experiment. The latter, however, gave slightly lower values than the basal diet + $KH_2PO_4$ + cod liver oil.

The rats grew well on the rachitic diet, but in most cases growth was not as good as on the rachitic diet + $KH_2PO_4$. The latter, however, did not give as good growth as the rachitic diet $+KH_2PO_4$ + cod liver oil.

## 4681

### Liver Extract Therapy in Splenectomized Anemic Rats.

HARRIS B. SHUMACKER, JR., AND LYDIA BOWMAN EDWARDS.
(Introduced by Florence R. Sabin.)

*From the Department of Anatomy, Johns Hopkins University.*

The therapeutic effect of liver extract on splenectomized anemic rats was investigated with the principal object of inquiring into the possibility of developing a biological assay for liver extracts. Great similarity between this severe rat anemia and human pernicious anemia had been noted by several observers, Lauda going so far as to name the disease "perniziöse Anämie" in rats.[1] At the time of our research we were unaware of the recently completed work of Vedder, who found that liver extract therapy was of no value in the treatment of this anemia.[2]

Our experiments confirmed this finding. In a series of 40 splenectomized rats (averaging 40 gm. in weight, the experimental animals being fed from 0.0125 gm. to 0.4 gm. of Lilly's liver extract No.

[1] Lauda, *Klin. Woch.*, 1925, ii, 1587.

[2] Vedder, A., *Nederl. Tijdschr. v. Geneesk.*, 1928, ii, 4411.

343 per day) no effects of the extract were noted as judged by the mortality rate, length of life after splenectomy, and total erythrocyte count. Fifty-seven per cent of the experimental animals died in a period of from 6 to 26 days after splenectomy, average 15 days; 60% of the controls died in a period of from 5 to 25 days after splenectomy, average 13 days. No significant differences were noted in the erythrocyte counts of the control and experimental animals.

4682

## Studies on the Physiology of Pyrimidines. The Metabolism of the Pyrimidine Nucleosides.

O. H. EMERSON AND L. R. CERECEDO. (Introduced by Carl L. A. Schmidt.)

*From the Division of Biochemistry, University of California Medical School, Berkeley.*

The results of previous work by one of us[1] indicated that the pyrimidines uracil and thymine, when fed in small amounts to dogs, are to a large extent metabolized. In the case of cytosine it was found that this substance was not utilized. It was partly excreted unchanged, partly deaminized and excreted in the form of uracil.

These experiments were undertaken to study the behavior of the pyrimidine nucleosides, uridine and cytidine. The substances were fed to dogs maintained on a nitrogen equilibrium. Our results show that these nucleosides are metabolized in the animal body, being broken down predominantly to urea. The behavior of cytosine in the nucleoside molecule is therefore in marked contrast to that of the free base, which is not utilized. Pentose determinations in the urine showed that the pentose sugar in the nucleoside molecule is practically completely burned; certainly not more than a trace is excreted in the urine.

Working with rabbits and man Wilson[2] previously concluded that when uracil, in the form of a nucleoside or nucleotide, is administered, only a small portion of the uracil is excreted.

[1] Cerecedo, L. R., *J. Biol. Chem.*, 1927, lxxv, 661.

[2] Wilson, D. W., *J. Biol. Chem.*, 1923, lvi, 215.

## 4683

### Are There Bacterial-Protein Hybrids?

BECKY B. VEBLEN. (Introduced by W. H. Manwaring.)

*From the Laboratory of Bacteriology and Experimental Pathology, Stanford University, California.*

Fourth generation of subcultures of *B. typhosus, S. viridans,* and certain other microorganisms grown in 10% horse serum (Ringer's solution) are agglutinated in dilutions of 1:20 to 1:160 by anti-horse rabbit precipitin, controls cultures (nutrient broth) showing no agglutination.

With eighth to twelfth generation subcultures of the same microorganisms, definite agglutination takes place with 1:1000 anti-horse rabbit precipitin. The susceptibility of eighth to twelfth generation subcultures to precipitin-agglutination is not materially reduced by repeated washings with Ringer's solution. No agglutination of these subcultures takes place with any heterologous precipitin thus far tested (*e. g.,* anti-egg rabbit precipitin).

## 4684

### Effects of Anterior Pituitary Extract Upon an Hypophysectomized Puppy.

FREDERICK LEET REICHERT.

*From the Halsted Laboratory of Experimental Surgery, Stanford University Medical School.*

Gigantism has been produced by Evans and Long[1] by the injections of an anterior pituitary extract in immature rats. The same result was obtained by Putnam, Teel and Benedict[2, 3] when the extract was administered to a bulldog. Normal growth was induced by injections of this fluid in hypophysectomized rats although there was no evident repair of the thyroid, suprarenals or gonads.[4] Putnam, Teel and Benedict[2] state that "in hypophysectomized dogs and

[1] Evans, H. M., and Long, J. A., *Anat. Rec.*, 1921, xxi, 62.

[2] Putnam, T. J., Teel, H. M., and Benedict, E. B., *Am. J. Physiol.*, 1928, lxxxiv, 157.

[3] Putnam, T. J., Benedict, E. B., and Teel, H. M., *Arch. Surg.*, 1929, xviii, 1708.

[4] Smith, P. E., *J. Am. Med. Assn.*, 1927, lxxxviii, 158.

rats restoration of growth has been produced in preliminary experiments."

The effect of the anterior pituitary extract upon a hypophysectomized puppy has been studied over a period of 2½ months. A sterile bovine extract of the anterior lobe, the preparation of which is given elsewhere,[5] was kindly furnished by Dr. H. M. Evans of the University of California. This extract has been found active for the growth hormone when administered to rats.

By means of the intracranial approach of Dandy and Reichert[6] a total hypophysectomy was performed on an 8 weeks old female puppy. That a total extirpation was accomplished was indicated when the animal remained infantile and failed to grow over a period of 4 weeks. During this period the increase in length of both the femur and tibia, measured by skiagrams, was 6 mm. and of the skull 2 mm., while in the litter mate control the femur and tibia had lengthened 33 mm. and the skull 12 mm. The weight of the operated puppy increased 32 gm. while the control gained 88 gm.

Daily intraperitoneal injections (25 cc.) of the anterior pituitary extract were given during the following 2½ months. At the end of this period the operated puppy although quite thin appeared in size the same as the control. During these 10 weeks the length of the femur and tibia of the control increased 53 mm. and of the skull 10 mm., while the treated hypophysectomized puppy showed a gain of 70 mm. in the length of the femur and tibia and 24 mm. in length of skull.

The weight curve of the treated puppy paralleled that of the control in spite of the fact that the animal suffered from diarrhoea and vomited after each injection. The treated puppy gained 164 gm. and the control 222 gm. during that period.

After 4 weeks of treatment permanent teeth appeared and replaced the milk teeth in the following month. At the end of the period of 2½ months permanent teeth were just appearing in the control.

No appreciable change has been noted to date in the appearance of the coat of hair or of the external genitalia.

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[5] Evans, H. M., Cornish, R. E., and Simpson, PROC. SOC. EXP. BIOL. AND MED., 1929, xxvii, 101.

[6] Dandy, W. E., and Reichert, F. L., *Bull. Johns Hopkins Hosp.*, 1925, xxxvii, 1.

## 4685

### Effect of Endothelial Blockade on the Rate of Intravenous Denaturization of Foreign Proteins.

T. H. BOONE. (Introduced by W. H. Manwaring.)

*From the Laboratory of Bacteriology and Experimental Pathology, Stanford University, California.*

Intravenously injected horse proteins are retained quantitatively in the normal canine circulation for at least 4 days, by which time they are so far denatured as to call forth no recognizable anaphylactic reaction on massive blood transfusion into horse-protein-hypersensitive recipients.[1]

No anaphylactic denaturization is demonstrable at the end of 4 days in endothelial blockaded (India ink) dogs. Approximately half of the routine protein dose remains anaphylactically active as late as the ninth day in these animals, and about an eighth as late as the fourteenth day.

This paper summarizes the results from 20 transfusion tests. The technique has been previously described.[1]

## 4686

### A Study of the Bacterial Flora of Organs and Body-Fluids at Necropsies.

CASPAR G. BURN. (Introduced by Raymond Hussey.)

*From the Division of Pathology, Yale University, New Haven.*

During the past year a careful systematic bacteriological study has been made upon the organs and body-fluids that were obtained from 168 necropsies. This communication is a preliminary statement concerning the results obtained and the technique employed.

The technique for the collection of the material from the organs and body-fluids consists of carefully searing the surface of the organ or vessel and removing a portion of the tissue. The material is immediately placed in a sterile receptacle and removed to the laboratory where various kinds of media are inoculated. The media used routinely for every organ and fluid is (1) a surface infusion agar plate that contains 5% rabbit blood; (2) infusion broth; (3) Hol-

[1] Manwaring, W. H., *et al.*, *J. Immunol.*, 1927, xiii, 357, and 1928, xv, 351.

man's Meat Media and (4) Sabouraud's agar. In addition, for each fluid specimen obtained, 2 pour agar plates are prepared for the purpose of a quantitative estimation of the organisms present. Direct smears that have been stained by a special bacteriological stain have recently been added as a routine procedure for every organ or fluid cultured. This is to serve as a microscopic check for the cultured findings.

The bacteriological studies were made upon necropsies from many different pathological conditions, including deaths from acute and chronic causes and several deaths due to accidents. There was, in 75% of necropsies, an average of one to 5 hours elapsing between the time of death and the time of culturing. Very few necropsies had an interval of 24 to 48 hours before culturing. Of these, it is surprising to find that the greater portion were free of organisms.

TABLE I.

*Frequency of Occurrence of Bacteria in Organs and Body-fluids in 168 Necropsies.*

| Name of organ or body-fluid | Total number of cultures | Per cent positive for bacteria |
|---|---|---|
| Bronchi | 100 | 100 |
| Lungs R | 122 | 95 |
| Lungs L | 94 | 94 |
| Kidney | 109 | 83 |
| Pleural fluid | 30 | 83 |
| Liver | 124 | 77 |
| Pericardial fluid | 40 | 73 |
| Spleen | 117 | 71 |
| Heart blood | 148 | 57 |
| Peritoneal fluid | 47 | 55 |
| Spinal fluid | 84 | 56 |
| Urine | 60 | 50 |

TABLE II.

*The Frequency of Occurrence of Bacteria Isolated from 168 Necropsies.*

| Type of Organism | Total Number of Necropsies Positive | Per cent of Necropsies Positive |
|---|---|---|
| *Staphylococcus—aureus or albus* | 101 | 60 |
| *Bacillus coli* | 91 | 54 |
| *Streptococcus—non-hemolyticus or viridans* | 71 | 42 |
| *Streptococcus hemolyticus* | 55 | 33 |
| *Bacillus influenzae* | 47 | 28 |
| *Micrococcus zymogenes* | 31 | 18 |
| Pneumococcus Type IV | 26 | 15 |
| Diphtheroids | 18 | 11 |
| *B. lacti-aërogenes* | 17 | 10 |
| *Bacillus proteus* | 10 | 6 |
| Pneumococcus Type III | 9 | 5 |
| Pneumococcus Type I | 7 | 4 |
| Pneumococcus Type II | 6 | 4 |
| *Bacillus Friedländer* | 5 | 3 |

TABLE III.

*Frequency of Occurrence of Bacteria Isolated from Each Organ and Body-fluid.*

| | Name and Total Number of Organs and Body-fluids Cultured. | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Heart blood | Pericardial fluid | Lungs | | Bronchi | Pleural fluid | Liver | Bile | Spleen | Kidney | Urine | Peritoneal fluid | Spinal fluid |
| | | | Right | Left | | | | | | | | | |
| | 148 | 40 | 122 | 94 | 100 | 30 | 124 | 75 | 117 | 109 | 60 | 47 | 84 |
| | % | % | % | % | % | % | % | % | % | % | % | % | % |
| *Staphylococcus aureus* or *albus* | 11 | 23 | 40 | 47 | 52 | 33 | 14 | 11 | 15 | 21 | 15 | 21 | 19 |
| *Bacillus coli* | 14 | 20 | 36 | 26 | 25 | 0 | 27 | 16 | 19 | 38 | 23 | 21 | 8 |
| *Streptococcus non-hemolyticus* or *viridans* | 6 | 10 | 27 | 21 | 32 | 17 | 10 | 3 | 9 | 13 | 3 | 11 | 4 |
| *Streptococcus hemolyticus* | 9 | 15 | 21 | 24 | 26 | 10 | 10 | 0 | 13 | 9 | 2 | 6 | 6 |
| *B. influenzae* | 1 | 3 | 22 | 30 | 32 | 7 | 2 | 0 | 1 | 0 | 0 | 2 | 2 |
| Pneumococcus | | | | | | | | | | | | | |
| Type I | 3 | 5 | 5 | 6 | 3 | 7 | 2 | 0 | 1 | 0 | 0 | 2 | 1 |
| Type II | 3 | 0 | 4 | 5 | 4 | 7 | 2 | 0 | 2 | 3 | 0 | 0 | 0 |
| Type III | 3 | 0 | 4 | 5 | 6 | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 5 |
| Type IV | 4 | 3 | 17 | 14 | 17 | 10 | 2 | 0 | 3 | 2 | 0 | 0 | 4 |
| *B. lacti-aërogenes* | 3 | 0 | 6 | 4 | 5 | 3 | 3 | 5 | 5 | 6 | 5 | 2 | 1 |
| *B. Friedländer* | 0 | 3 | 2 | 3 | 3 | 0 | 1 | 1 | 1 | 0 | 2 | 0 | 0 |
| *B. proteus* | 1 | 0 | 7 | 2 | 3 | 3 | 5 | 1 | 5 | 4 | 2 | 0 | 1 |
| Diphtheroid | 1 | 5 | 3 | 2 | 2 | 0 | 3 | 0 | 4 | 5 | 0 | 0 | 0 |
| *M. zymogens* | 5 | 3 | 2 | 2 | 1 | 0 | 7 | 8 | 6 | 3 | 5 | 0 | 4 |

However, it was found that if there were 7 to 9 hours elapsing between time of death and time of culturing there would be an increase in the frequency of the occurrence of anaerobic organisms within the tissues. The organism that predominated was a large gram positive spore-forming rod which resembles *Bacillus sporogenes* in many of its cultural characteristics. Yeasts and molds were absent from the cultures, except from lungs and bronchi. From this source, the green mold was commonly present.

No attempt will be made at this time to offer an interpretation of the results presented above. The work is being continued and it is our hope that after a larger number of necropsies have been investigated, we may be able to draw some significant conclusions.

## 4687

## The Effect of Roughage Upon Growth.

C. M. MCCAY. (Introduced by L. A. Maynard.)

*From the Department of Animal Nutrition, Cornell University.*

The diets shown in Chart 1 were designed for the purpose of determining the influence of large quantities of "roughage" upon the rate of growth. Ration 1 contains 10% cellulose while numbers 2 and 3 contain 20%. Diet 4 contains 20% of agar-agar. Nos. 1 and 2 contain moderate amounts of fat while numbers 3 and 4 have 45% of their total weight in the form of lard and cod liver oil. All rations were designed to contain adequate amounts of protein, fats, carbohydrates, mineral matter and vitamin supplements. The levels for protein and mineral matter were increased for rations 3 and 4 in accordance with the procedure of Smith and Carey.[1] The salt mixture employed was that of Osborne and Mendel. Cellophane was selected as a source of cellulose since it represents a very pure form. In addition to the mixed diet all animals were fed separately a daily allowance of 200 mg. of vitavose and 3 drops of cod liver oil.

Ten male rats were reared from the time of weaning upon rations 1 and 2. These showed a marked decreased rate of growth when compared with a control group whose growth rate is shown by curve C while representative rates for the other groups are shown by typical curves properly numbered. The control ration was iden-

[1] Smith, A. H., and Carey, E., *J. Biol. Chem.*, 1923, lviii, 425.

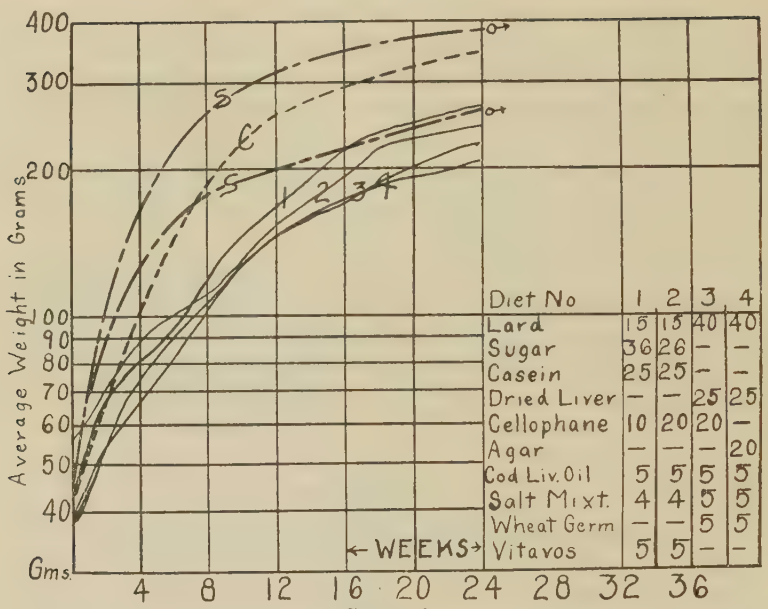


CHART 1.

Growth curves of rats reared upon high roughage diets. Semi logarithmic scale diagram.

tical with number 1 except that the cellulose was replaced by the same per cent of sucrose. Curves S are those for males and females reared upon our stock ration. These are described elsewhere by Maynard.[2] Since we obtained subnormal growth with rations 1 and 2 containing 10 and 20% cellulose we felt that this might be due to the inability of the rat to consume sufficient calories. The high calorie diets Nos. 3 and 4 were designed for subsequent experiments. Five male and 5 female rats were reared upon each of the high fat rations. Both sexes showed identical growth rates. Hence, the females were much less affected than the males. We must conclude that growth of rats remains subnormal upon high roughage diets independent of whether the diet contains a moderate or a large amount of fat.

No evidence of physical injury by diets containing large amounts of roughage has been obtained. With 3 exceptions all rats reared upon these rations have attained maturity and except for being slightly smaller cannot be distinguished from our best stock animals. All animals were kept for more than 10 months with no other diet than these respective rations. Those upon rations 1 and 2 were

[2] Maynard, L. A., *Science*, in press.

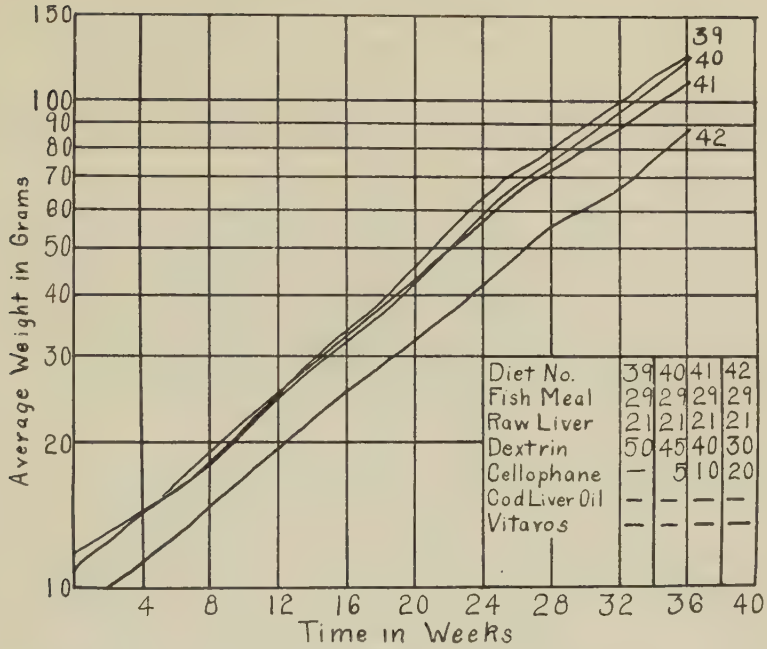


CHART 2.

Growth curves of brook trout reared upon diets with varying amounts of cellulose. Semi logarithmic scale diagram.

kept upon the diets for more than 13 months. Little difference was noted between those fed cellulose and those fed agar-agar.

In Chart 2 we have shown representative growth curves for brook trout reared upon rations containing 5, 10 and 20% of cellulose in rations 40, 41 and 42 respectively in comparison with diet 39 which contains none. Thirty trout were fed each of the experimental rations. The growth curves represent the rates for the average individuals of each group. The growth curves are logarithmic and show rates which are comparable with the best we have obtained under any dietary conditions. The same technique of feeding and weighing was employed as that described previously.[3] From these data one must conclude that trout in spite of their very short intestinal tract can tolerate very large amounts of roughage in their rations without any influence upon their rate of growth.

[3] Titcomb, J. W., *et al., Trans. Am. Fisheries Soc.*, 1928, lviii, 205.

4688

## A Study of Continued Alkalinity of Morning Urine.

ROGER S. HUBBARD AND CATHERINE B. ALLISON.

*From the Laboratory of Clifton Springs Sanitarium and Clinic, Clifton Springs, New York.*

A series of determinations of the reaction of successive specimens of morning urine has been carried out on 296 subjects, most of whom were studied because an absence of hydrochloric acid in the gastric juice was suspected. There were twenty-two of these, in whom all of the 5 or 6 specimens obtained, gave pH values 7.0 or higher. It has seemed worth while to try to determine the cause of such a finding, particularly as duplicate studies on the same patient usually gave results which were similar.

The case histories were reviewed, and nothing significant found in the diagnosis or in the age and sex to distinguish these patients from the rest of the series. The group was remarkably free from signs of nephritis. Only one patient had cystitis. Diagnoses of neuroses of one type or another, and of various forms of gastro-intestinal disorders were frequent, but probably not more frequent than can be explained on the basis of the type of subjects who were submitted to the test. The only finding distinguishing this group was the small number of patients who showed an absence of hydrochloric acid in the gastric juice. There were only 2 of these included, while approximately a third of the whole series gave such a finding.

The average figures showed two things. First, the reaction of the night specimen was decidedly less alkaline than was that of those collected during the morning. As a matter of fact, in 13 of the 20 cases where the necessary data were available this specimen was less alkaline than that collected immediately after awakening, and in 7 of these 13 the pH was actually lower than 7.0. This suggests that the morning alkalinity in a fair proportion of these cases was not due to disease (cystitis) or to the diet taken before the test because in these conditions there is also an alkalinity of the night specimen. Respiratory adjustment to waking conditions seems to be the most satisfactory explanation of the development of alkalinity immediately after awakening before any meal is fed.

The second thing noticeable about the average figures is that, while the difference among the reactions of the morning specimens was small, the results assumed the form of a curve with 2 periods of alkalinity. The second one—after the meal—may be ascribed to

the secretion of hydrochloric acid in the stomach, while the first one, which has its highest pH value in the first hour, is probably due to respiratory adjustment.[1] In one case of achlorhydria, it was possible to alter the form of the curve markedly by keeping the patient in bed, while a similar experiment upon a normal subject caused no such change in the form of the results obtained. The difference between the results could be adequately explained by the presence of changes in the body fluids in the normal patient which were absent in the case of achlorhydria. The factor of adjustment to waking conditions is common to both, and is lessened by the stay in bed when respiratory variations are reduced to a minimum. In the normal patient, however, the secretion of acid may cause changes which make further demands on the regulatory mechanism of the organism.

The proof that such an explanation is adequate for the experiment upon the normal subject cannot be regarded as complete, for the result was the same when a meal was taken and when it was omitted. This has been observed in some, but not all, normal cases[2] and is difficult to explain on a basis of gastric secretion. The afternoon curve upon the normal subject was regular; and showed that she then responded in the usual manner to the secretion of acid by the stomach.

*Conclusion:* A fairly large number of persons show a marked urinary alkalinity which persists through the morning period. This alkalinity may be independent of diseases of the kidneys and urinary tract, and of the type of diet preceding the test. It may not be present during the afternoon period. It probably represents an unusual adjustment to waking conditions, exaggerated in most instances by the secretion of hydrochloric acid by the stomach. The reason why such an adjustment is different in these cases from that more commonly observed did not appear in a study of the type of patient upon whom the results were obtained. It seems to the authors that the following general statement covers the fact: there are 2 methods of compensating for a tendency towards alkalosis—through the urine and through the lungs. In these subjects the compensation is accomplished through the kidneys to a greater extent than is the case in most persons.

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[1] Hubbard, R. S., *J. Biol. Chem.*, 1929, lxxxiv, 191.

[2] Hubbard, R. S., and Steele, T. M., *J. Biol. Chem.*, 1929, lxxxiv, 199.

4689

## Longevity of Bact. Tularense in Muscle Tissue.

R. G. GREEN AND E. M. WADE.

*From the Department of Bacteriology and Immunology, University of Minnesota, and State Department of Health, Minneapolis.*

As the result of investigations carried out by Francis, it has become customary to test for the presence of *Bact. tularense* in animals dead from tularemia by the injection of a suspension of liver or spleen into guinea pigs. Francis[1] has pointed out that *Bact. tularense* gradually disappears from the liver and spleen of animals dead from tularemia. He[2] has demonstrated that *Bact. tularense* in spleen tissue may be kept viable 30 days or longer by storage in 50% glycerine at ice-box temperature. He states that liver is inimical to the life of the infection and, when stored in glycerine with spleen, will destroy the infectivity of the spleen tissue. In his investigation of rabbit livers from the Washington Market, Francis[3] found *Bact. tularense* viable in rabbits shipped from Tennessee, but the lapse of time after death was unknown.

We have investigated the longevity of *Bact. tularense* in the liver and spleen of animals dead from experimental tularemia and have gathered comparative data with respect to the longevity of the organism in muscle tissue. Tissues from one guinea pig and 2 rabbits were injected at intervals into 142 guinea pigs used as test animals.

*Test No. 1.* The carcass of a guinea pig dead from experimental tularemia 4 days after inoculation was kept at room temperature (ca. 20°C.). The spleen and liver were thickly studded with necrotic areas. At intervals portions of the liver and spleen of approximately the same size were removed and emulsified in normal saline solution. The suspensions were immediately injected subcutaneously into guinea pigs. The results are shown in Table I.

*Test No. 2.* The carcass of a rabbit dead from experimental tularemia was used. The rabbit died 4 days after inoculation, with both inguinal glands enlarged and the liver and spleen well covered with necrotic areas. The carcass was stored at 6°C. with the organs *in situ*. At intervals given below, small pieces of liver, spleen and muscle were removed and weighed. Two suspensions were made, containing 0.1 gm. tissue per cc. and 0.01 gm. tissue per cc., respec-

[1] Francis, Edward, Bulletin No. 130, Hygienic Laboratory, 1922, 6.

[2] *Ibid., J. Am. Med. Assn.*, 1928, xci, 1155.

[3] *Ibid., J. Am. Med. Assn.*, 1925, lxxxiv, 1243.

TABLE I.

| Duration of storage | Guinea pigs inoculated | Liver | Spleen |
|---|---|---|---|
| Fresh | 2 | + | Not done |
| 2 days | 2 | + | Not done |
| 3 " | 1 | + | + |
| 4 " | 1 | — | — |
| 5 " | 1 | — | + |
| 6 " | 1 | — | — |
| 7 " | 1 | — | Uns. |
| 9 " | 1 | — | Uns. |

+ Guinea pig died with lesions typical of tularemia.
— Guinea pig chloroformed after at least 3 weeks; no evidence of tularemia.
Uns.—"Unsatisfactory." Guinea pig died of septicemia within 36 hours.

TABLE II.

| Duration of storage in icebox | Liver | | Spleen | | Muscle | |
|---|---|---|---|---|---|---|
| | 0.1 gm | 0.01 gm. | 0.1 gm | 0.01 gm. | 0.1 gm | 0.01 gm. |
| Fresh | + | + | + | + | + | + |
| 3 days | + | + | + | + | + | + |
| 5 " | + | + | + | + | + | + |
| 7 " | + | + | + | + | + | + |
| 8 " | — | — | + | + | Not done | |
| 10 " | — | — | — | — | + | + |
| 12 " | — | — | — | — | + | + |
| 24 " | | | | | + | + |
| 31 " | | | | | — | — |
| 33 " | | | | | + | — |
| 36 " | | | | | — | — |

tively. Much difficulty was experienced in suspending the muscle tissue in normal saline solution, and this was imperfectly accomplished. The suspensions were injected immediately after preparation. The results obtained are found in Table II.

*Test No. 3.* A rabbit carcass was stored at 6°C. under the same conditions as the carcass in Test No. 2. This rabbit died 4 days after inoculation, with left inguinal gland enlarged and the liver and spleen thickly studded with necrotic areas. Liver, spleen and muscle tissue suspensions were injected into guinea pigs in amounts of 0.1 and 0.01 gm., prepared as above. The results of this experiment are shown in Table III.

While these results are obtained from 3 carcasses only, they give some indication as to the persistence of the infection in the tissue of a dead rabbit or guinea pig. They show a remarkable persistence of the viable organism in muscle as compared with liver and spleen. Consistent results observed for muscle tissue during the first 24 days, indicate that the variable results observed for liver and spleen are dependent upon actual changes taking place in the tissue. Inasmuch as negative results were obtained as early from the spleen as

TABLE III.

| Duration of storage in icebox | Liver | | Spleen | | Muscle | |
|---|---|---|---|---|---|---|
| | 0.1 gm. | 0.01 gm. | 0.1 gm. | 0.01 gm. | 0.1 gm. | 0.01 gm. |
| Fresh | + | + | + | + | + | + |
| 2 days | + | + | + | + | + | + |
| 3 " | + | + | + | + | + | + |
| 4 " | + | + | + | + | + | + |
| 5 " | + | + | Uns. | + | + | + |
| 6 " | + | + | + | — | + | + |
| 8 " | — | — | — | — | + | + |
| 10 " | + | + | None left | | + | + |
| 12 " | — | — | | | + | + |
| 14 " | Uns. | + | | | + | + |
| 17 " | — | — | | | + | + |
| 20 " | — | — | | | + | + |
| 22 " | | | | | + | + |
| 24 " | | | | | + | + |
| 29 " | | | | | + | + |
| 33 " | | | | | + | — |
| 35 " | | | | | + | — |
| 38 " | | | | | — | — |
| 42 " | | | | | — | — |
| 46 " | | | | | — | — |

from the liver, no marked difference in the viability of *Bact. tularense* in liver and spleen tissue of rabbits is discernible from our data. These tissues may not be infective on the eighth day after death when stored at 6°C. It appears that rabbit muscle, under the same conditions, will retain its infectivity consistently for about 4 weeks and may be infective for as long as 35 days.

## 4690

## Effect of Changes in Environment on Development of the Chick.

L. K. MUSSELMAN. (Introduced by H. S. Burr.)

*From the Departments of Anatomy and Obstetrics and Gynecology of the School of Medicine, Yale University.*

The work of Stockard in varying the normal environment of developing eggs with resulting malformations suggested carrying out similar experiments on a warm blooded animal, such as the chick. The eggs were placed in an incubator for 16 to 18 hours prior to operation. A piece of shell was removed, and in some instances 3 drops of 1/1000 solution of 95% alcohol were added to the side of the embryo, in others, 3 drops of normal saline (both at room and incubator temperatures), while a few eggs were carried along as controls.

At various times, chiefly 48 and 72 hours later, the eggs were reopened and where embryos existed, they were fixed. Photographs were made and then the specimens were sectioned. The control eggs showed no abnormality.

*Results:* Total eggs treated with saline _____18
Total eggs treated with alcohol_____27

Of these 5 were completed for microscopic study; of them all fail to show a pituitary gland; 4 showed optic defects; 2 showed failure of closure of the head folds and in one no olfactory pits appeared. It made no apparent difference in the results whether the environment was changed with weak alcohol or normal saline solution. The treated embryos exhibited malformations of the nervous system. The abnormalities were chiefly (1) an absence of the pituitary gland, (2) in some an absence of an eye, (3) failure of the head folds to close, (4) in one, an absence of the olfactory pits.

4691

## Relative Value of Splanchnic and Spinal Analgesia in Treatment of Experimentally Produced Ileus.

ALTON OCHSNER, I. M. GAGE AND R. A. CUTTING.

*From the Department of Surgery, School of Medicine, Tulane University, New Orleans, La.*

In a previous communication[1] we reported the results of splanchnic analgesia in the treatment of experimental ileus, and concluded that this form of treatment is undoubtedly of great value in reestablishing motility in the intestinal wall. The present communication concerns the relative value of splanchnic analgesia and spinal analgesia. Our results are based upon a series of 70 dogs. In 50 animals novocain splanchnic analgesia was produced, and records of the blood pressure, intestinal motility, and respirations were obtained on the kymographic drum. In most of the cases a single tracing of intestinal motility was obtained, and this from the terminal ileum. In some cases, however, additional tracings were obtained from the duodenum and also from the colon. In 9 animals instead of using novocain an aqueous solution of nicotine was used, as advocated by

[1] Ochsner, A., Gage, I. M., and Cutting, R. A., *J. Am. Med. Assn.*, 1928, xc, 1847.

Rosenstein and Köhler.[2] Nicotine is apparently an unsatisfactory substance when used in this way, since it increases the blood pressure unduly, and fails to increase the intestinal motility to a satisfactory degree. The range of blood pressure rise in all but 3 animals was greater than 90 mm. of mercury, and, while in 5 cases the tone in the ileum was raised from 10 to 50 mm., as recorded by the writing point on the kymographic drum, the other 4 animals showed insignificant effect on both tone and motility, and in the case of the duodenum and the colon the tone of the intestine was actually decreased.

The results of the present investigation, with respect to novocain, show that both the tone and amplitude of intestinal movement are markedly increased in the ileum, the average figure for the tone being an increase of 29.5 mm. and in the amplitude a value of 11.2 mm. The induction of novocain splanchnic analgesia, however, reduces the blood pressure, but not unduly, the average figure being 20 mm. of mercury. The duration of novocain splanchnic analgesia is relatively short, averaging 5½ to 6 minutes, but is sometimes much longer. Spinal analgesia was induced in 13 animals, and while the effect in general was similar to that produced by novocain splanchnic analgesia, the fall in blood pressure was much more marked, and the effect on the intestinal tone and motility was relatively inconsiderable. The fall in blood pressure, when using spinal analgesia, is from 2 to 3 times as great as results from the injection of novocain analgesia, and the effects on tone and intestinal movement tend to be inconstant and slight. In connection with both the splanchnic and spinal analgesia, the administration of ephedrin and adrenalin, in order to combat the blood pressure depression incident to the use of these two methods, was found to negative the effect of the method in that when either of these two drugs was used the effect of the analgesia was not noticeable at all or at least very slight. The explanation seems to be that both of these drugs have a direct action in relaxing the intestinal musculature.

The rationale of the treatment of ileus by the induction of both spinal and splanchnic analgesia depends upon the conception of the dual innervation of the intestine by vagus and splanchnic nerve fibers. These 2 systems of nerves are conceived to be essentially antagonistic, a stimulation of the vagus nerve producing a motor effect while stimulation of the splanchnic nerve produces an inhibitory effect. Any procedure, therefore, which tends to prevent impulses from reaching the intestine by way of the splanchnics tends to render in-

[2] Rosenstein, P., and Köhler, Hans, *Deut. Z. f. Chir.*, 1928, 210. *Idem, Med. Klin.*, 1926, xxii, 530.

operative the inhibitory system and to leave the motor supply in full control. Thus does section of the splanchnic nerve tend to increase motility. The induction of both spinal and splanchnic analgesia is the method of producing chemical section of the splanchnic nerve fibers. Spinal analgesia produces such section of the white *rami communicantes* as they leave the spinal cord. Splanchnic analgesia interrupts the fibers of the splanchnic nerves at the point at which they break up into the splanchnic plexuses anterior to the bodies of the first and second lumbar vertebrae. Nicotine splanchnic analgesia interrupts the synapses which occur in the semilunar ganglia, that is, the connection between the pre and post ganglionic fibers of the splanchnic nerves. Possibly the explanation for the relative insignificant effect of nicotine is that the drug cannot be deposited in the semilunar ganglia themselves. Novocain splanchnic analgesia, however, is much more effective, since this drug acts upon the nerve fibers themselves, and consequently interruption of impulses occurs whenever the saturation of the solution reaches a sufficient value in the region of the nerve filaments. Spinal analgesia should be as effective as splanchnic analgesia if all the fibers entering into the formation of the splanchnic nerves, or at least the reflex involved in the splanchnic control of the intestine, were blocked. The spinal analgesia is actually not as efficient as novocain splanchnic analgesia, seems to indicate that a part of the reflex involved in the inhibitory regulation of intestinal movement occurs by way of a reflex arc which does not traverse the spinal cord. Such effect that spinal analgesia has on intestinal motility seems to be somewhat more prolonged than does the effect produced by splanchnic analgesia, and the explanation for this is not clear.

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## Relative Values of Heat and Cold on Experimentally Produced Peritonitis.

ALTON OCHSNER, I. M. GAGE, R. A. CUTTING AND EARL GARSIDE.

*From the Department of Surgery, School of Medicine, Tulane University, New Orleans.*

Experiments were undertaken to throw light on the controversial question of the relative value of local applications of heat and cold on the course of peritonitis. They are based on 119 animals. Of these, 9 were sacrificed in obtaining cultures.

Experimental peritonitis was produced in dogs by the intraperitoneal injection of combined anaerobic and aerobic cultures obtained from animals in which a primary peritonitis occurred as a result of artificially produced obstruction of the cecal appendage. Peritoneal fluid was obtained with hypodermic syringe and needle and inoculated into tubes of meat digest broth, one test tube being incubated under anaerobic and the other under aerobic conditions. The 24 hour cultures of the 2 tubes were then mixed, and equal quantities of the resultant mixture introduced into the peritoneal cavity by hypodermic needles, the skin having previously been shaved and sterilized. The injection was uniformly made into the left lower quadrant at a point approximately midway between the umbilicus and anterior superior spine of the ilium. The animals were then divided into 3 groups. One group was used as control, and received no treatment except the administration of hypodermoclysis. A second group received, in addition to hypodermoclysis, a Leiter coil over the area of injection, through which a continual stream of cold brine flowed. In the third group a similar application to the abdominal wall was made, but hot water was passed through the coil. Observations every 3 hours included rectal temperatures and temperatures of the coil, the latter being obtained by a thermometer inserted between the lower surface of the coil and the abdominal wall. Hemoglobin estimation and leucocyte and differential blood count were made every 24 hours during the survival of the animal. Animals which died were immediately subjected to autopsy, and a complete protocol of the abdominal findings was recorded.

Of the animals recorded in the final series there were 25 control, 25 in which heat was applied, and 25 in which cold was applied. Of the animals surviving there were 10 each in the heat and cold series and 8 in the control series. In other words, 15 in the heat and cold

series died, while 17 in the control series died. The average duration of life, computing the average number of hours lived, of the dogs which ultimately died was, in the heat series 40.4 hours, in the cold series 29.8 hours, and in the control series 22.9 hours. The animals of all series showed a marked leucocytosis at the end of 24 hours and the maximal leucocytosis was attained during the first 48 hours. Blood counts at the end of 72 and 96 hours showed a diminishing leucocytosis, but the animals treated with cold differed from both the control series and the dogs treated by heat in that a relatively high leucocytosis tended to persist even at the end of 96 hours, whereas in both the control and the heat series the leucocyte count at this time was considerably less than it had been normally. The hemoglobin estimation in all 3 series of animals is possibly of little significance, except as indicating the efficiency of the hypodermoclysis in preventing blood concentration. All the animals showed a polymorphonuclear leucocytosis at the end of 4 hours, and by the end of 48 hours this had become maximal. Estimations made at the end of 72 and 96 hours respectively showed diminution in the number of neutrophils, and at the end of 96 hours in all 3 animals the count had been reduced to a percentage considerably below normal. The dogs treated by heat showed a somewhat higher polymorphonuclear leucocytosis than either the animals constituting the control series or the animals treated by cold. The reaction of the small mononuclear cells consisted of a diminution in the count at the end of 24 hours in all 3 series, but the count progressively rose in all 3 series during the course of the succeeding 24 hour period up to the end of 92 hours, at which time the percentage of small mononuclear cells was considerably increased over what it had been originally. This effect was particularly noted in the series of animals treated by heat. Changes affecting the large mononuclear cells, the eosinophils, and the basophils were inconstant. In the technic used the temperature under the coil registered, in the case of the animals treated by heat, usually between 105° and 106° F. Underneath the coils of the dogs treated by cold the temperature fluctuated usually between about 74° and 79°. This fluctuation is obviously greater than that which occurred in connection with the heat coil. The average normal rectal temperature in the dog varied very considerably, some of the animals showing temperatures as low as 97° and others as high as 104°. The animals constituting the control series showed rectal temperatures ranging usually between 100° and 101°. Those treated by heat showed a temperature range of

between 101° and 102°; those treated by cold showed a temperature range of between 99° and 100°.

Results seem to indicate that the local application of heat and cold to the surface of the abdomen in the treatment of experimentally produced peritonitis is a matter of relative indifference. The raising or the lowering of the general body temperature by approximately a degree apparently has no influence on the ultimate survival of the animal, since as many animals survived when treated by heat as when treated by cold. It seems quite possible, however, that the application of heat or cold is of some value since a larger number of animals so treated survived than in the series untreated by either method.

4693

## Further Studies on the Pathogenicity of Br. Abortus and Br. Melitensis for Monkeys.

K. F. MEYER AND B. EDDIE.

*From the George Williams Hooper Foundation for Medical Research, University of California, San Francisco, California.*

The following observations have been made in the course of preliminary studies with 48 cultures of the brucella group on 74 rhesus and 14 cynomolgus monkeys:

A single oral administration of 21 different *Br. abortus* strains produced in 24 *Macacus rhesus* and 1 *M. cynomolgus* monkeys nonfebrile infections, followed by the formation of specific agglutinins of moderately high value. The dosage varied from 7 to 400 million and in some experiments it consisted of many billions. The strains identified serologically as abortus or para-abortus varieties and in the dye test as "bovis" or "melitensis" types had been isolated from bovine pathological specimens in the United States, Germany, Hungary, Italy and Switzerland.

Blood cultures have not been successful. The value of the serum agglutinins and their persistence depends on the feeding dose. Rapid disappearance of the agglutinative power to a low titer or to the zero point is worthy of note. A cutaneous application of approximately 20,000 bacteria has induced an infection. The incubation period as indicated by the appearance of the serum reaction varied from 9 to 30 days and is influenced by the infective dose.

The absolute evidence of infection has been secured through the recovery of the organisms from the tissues of 4 monkeys which have been sacrificed on the 34th to 52nd day. Three animals killed on the 43rd, 56th, 199th day furnished sterile cultures. Probably every *Br. abortus* strain when fed in sufficiently large dosage is pathogenic provided susceptible monkeys are used.

By feeding 100 million *Br. abortus* type "suis" of bovine, but in all probability of porcine origin which has retained its characteristics through the passage, a febrile disease with anatomical lesions indistinguishable from those of a *Br. melitensis* infection has been produced. During artificial cultivation the febrigenic properties on feeding have been lost but they have been retained when applied cutaneously. The milk of the cow which furnished one of the pathogenic "suis" strains has been consumed by a group of people without any bad effects.

An old laboratory culture of a *Br. abortus,* type "suis" of porcine origin infected via the alimentary tract when fed in large doses. The infection ran an afebrile course, stimulated after an incubation time of from 9 to 10 days a powerful agglutinative value of the serum with an abundance of specific organisms in the tissues.

A *Br. abortus* type "bovis" isolated from a swine foetus infected and immunized a monkey in a manner similar to that of the "bovis" types of bovine origin.

"Melitensis" strains of American origin possess a low virulence for monkeys; they may act like "bovis" cultures and they may lose their pathogenicity entirely within 6 months of artificial cultivation. Test-tube strains several years old are non-pathogenic and when administered by mouth they lack immunizing properties. One culture, which produced no lesions in guinea pigs by injection, infected a rhesus by mouth.

Tunisian strains of *Br. melitensis* fed or inoculated in doses of 100 million bacteria give rise to a febrile disease which is generally considered characteristic for this group of organisms. Even recently isolated strains may induce merely serologic but no febrile reactions.

A brucella organism serologically and biochemically an "abortus" and in its behavior towards dyes a "melitensis" type, acted like a typical melitensis by feeding and inoculation one month after isolation from a California case of undulant fever. In contrast 9 other strains kept under artificial cultivation for from 1 to 24 months and isolated from human abortus fever cases in Michigan, Iowa, North-

ern Germany and Denmark, infect monkeys when fed in a manner characteristic for the *Br. abortus,* "bovis" type.

Three *Br. abortus* type "suis" strains of human origin have not exhibited any striking pathogenicity or marked febrigenic properties neither by feeding nor by cutaneous or intravenous infection.

Serum agglutinins specific for the brucella group are formed only in the presence of a definite infection. The ingestion of heat killed abortus bacilli with or without bile is antigenically ineffective in monkeys and rabbits.

Over 10% of the rhesus and cynomolgus monkeys possess a natural immunity against brucella infections via the alimentary tract. Animals which react to the oral administration of virulent abortus organisms with moderate and in general transitory serum reactions resist subsequent feeding infections with *Br. abortus* "bovis" and "suis" but not with a Tunisian *Br. melitensis.* Continuous ingestion of small numbers of abortus may lead to mild, unrecognized or "silent" yet immunizing infections. At least in one observation, the local and general immunity thus induced has been definite.

## 4694

## Relation of Stainability and Electric Potential Differences to the pH Value.

R. BEUTNER* AND JOS. LOZNER.*

*From the Cleveland Clinic Foundation, Cleveland, Ohio.*

As shown previously[1] the addition of a water insoluble acid to a fat mixture leads to basophilic staining and a relative positive potential. The addition of a water insoluble base has the opposite effect on both properties. In tissues, basophilic staining is also associated with a positive potential, acidophilic staining with a negative potential. Water immiscibility of the acid or of an added base is essential. By the addition of a water-soluble acid or base both stainability and e.m.f. are influenced in opposite directions.

* Permanently associated with the Department of Physiology and Pharmacology of the University of Louisville.

[1] Beutner, R., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvii, 44.

An effect of this type is observed in Loeb's gelatin systems,† *e. g.*,

| | | | |
|---|---|---|---|
| saline + more alkal | (basophilic) sodium gelatinate on alkali. side of iso-electr. point | (acidophilic) gelatin chlorid on acid side of iso-electr. point | saline more acid — |

In this case also the basophilic substance is on the side of the positive pole; as a general rule we may consider that almost any system—

| | | | |
|---|---|---|---|
| + saline | basophilic substance or mixture | acidophilic substance or mixture | saline — |

produces an e.m.f. as indicated. A few exceptions to this rule have been observed in certain artificial systems which, however, are composed of substances not likely to occur in tissues in general and hence have a minor biological importance (details about these observations will be reported in a later publication).

According to a recent observation of R. Chambers[2] the nucleus has a higher pH value than the protoplasm indicating a slight alkalinity. This alkalinity, as well as the nuclear content of water-insoluble acids, probably bound to proteins as nucleo-proteins, would explain its basophilic stainability. Consequently, according to our experiments, the nucleus should be electrically positive as expected by G. W. Crile some time ago.

Whether the concomitant changes of stainability and e.m.f. are brought about in tissues chiefly by variations of its water-soluble or of its water-insoluble constituents can not yet be decided. Even if water-soluble constituents, or pH-changes play the most important rôle, this would not prove that proteins play a predominant rôle in the production of bioelectricity because the electromotive effect of Loeb's gelatin cell can also be produced by means of fats or other water-immiscible fluids.

To prove this point new experiments have been performed. A mixture of equivalent parts of a fatty acid and an oil-soluble amine in a neutral fat or other similar solvent was used. This mixture when shaken with aqueous buffer solutions of varying pH shows the

† This statement is derived from Loeb's "Proteins and Colloidal Behavior." On p. 28-30 he describes differential staining, and on p. 155 the electromotive forces of gelatin cells. It must be noted, however, that Loeb uses the + and — signs in the opposite sense for expressing the direction of the electromotive forces since he means by these signs the charge of the gelatin relative to the solution. The writer has formerly been misled by Loeb's presentation and quoted the sign of these gelatin cells and the corresponding oil cells in the wrong way in his lecture before the XIII International Physiological Congress, at Boston, August, 1929.

2 Chambers, R., *Biol. Bull.*, 1928, lv, 369.

same type of stainability as does gelatin, namely: staining acidophilic below a certain pH value; and basophilic above a certain pH value, forming the following cell system:

| acid | fatty acid amine mixture in oil | | alkal. |
|---|---|---|---|
| — buffer sol. | shaken with acid *acidophilic* | shaken with alkalin. sol. *basophilic* | buffer sol. + |

This shows that the negative pole is on the side of the acidophilic mixture in this case, just as in the case of gelatin and in olive oil mixtures with oleic acid or amylamine. Experimental details about this work will be published soon.

We see, therefore, that stainability and e.m.f. show the same relation in the case of proteins and of fats as well. It has not been possible so far to set up artificial systems composed of proteins which differ in their water-immiscible constituents, the reason being simply that proteins which dissolve a water-insoluble acid are not well known. We expect, however, to overcome this technical difficulty by combining in a cell system proteins containing preferably acid groups with those containing preferably basic groups. It is too early as yet to predict the outcome of such experiments, but if they are possible, they will very likely also reveal the same relation between stainability and e.m.f. which has been found in almost all other cases. *Hence none of our experiments prove that fats exclusively are the cause of bioelectricity.* Proteins might be used in the place of fats in every instance if the present technical difficulties can be overcome.

The writers wish to express their appreciation to Dr. G. W. Crile for his kind interest in this work.

4695

## An In Vitro Test to Indicate Basophilic or Acidophilic Character of a Dye.

R. BEUTNER* AND B. E. CAYWOOD.*

*From the Cleveland Clinic Foundation, Cleveland, Ohio.*

It has been found[1] that eosin, an acidophilic dye, is taken up by a fat mixture containing an oil soluble base, while a basophilic dye,

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[1] Beutner, R., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvii, 44.

methylene blue, dissolves in a fat mixture with an oil soluble acid. The fat mixture with a base is electrically negative against the mixture with an acid.

Considering the general character of these findings, it seems probable that the same conditions hold also in tissues; hence our model experiments are suitable for explaining that relation of stainability to electromotive forces which has thus far been found in tissues. To corroborate this finding we have performed the following comparative experiments. Forty-three different dyes were used for testing the differential stainability of artificial mixtures such as those mentioned which contain either oleic acid or an amine, and this was compared with the stainability of white blood cells. It was found in each case that a dye which was taken up, exclusively or preferably, by a non-aqueous solution of a higher fatty acid, produced a differential stain on the nucleus of white blood cells. On the other hand, any dye with a preference for a mixture containing a fat soluble base preferably stains the cytoplasm and leaves the nucleus unstained, or but slightly stained.

Many nuclear stains like methylene blue are water-insoluble bases. Evidently, they are taken up by a solution of oleic acid in a suitable solvent, forming, *e. g.,* methylene blue oleate, which is oil-soluble and hence stains. However, there are also nuclear stains which are not bases, *e. g., hematoxylin,* a widely used nuclear stain which has all the characteristics of an acid. It was of special importance to test the type of oil solubility in this case. One might have expected that the acidic hematoxylin was preferably soluble in an amine or alkaloid oil mixture, like other acid dyes, *e. g.,* eosin. Of course, this would have been in disagreement with its biological staining power, and would have tended to show the uselessness of our oil mixture as a biological standard of comparison. The experiment showed that this expectation was not correct. Hematoxylin was found to be taken up by the oleic acid oil mixture and not by the amine mixture. Hence, in this case too, the test tube experiment with oil mixtures furnishes decisive evidence as to the biological staining power; one could foretell that hematoxylin must be a nuclear dye in spite of acidic properties in aqueous solution. Possibly hematoxylin has basic properties in oil solution.

4696

## Relation Between the Isoelectric Range of Urease and the Reversibility of Its Action.

JOHN M. WILCOX. (Introduced by Carl J. Wiggers.)

*From the Department of Physiology, Western Reserve University Medical School, Cleveland, Ohio.*

The action of urease upon solutions of urea and ammonium carbonate was determined at various $H^+$ ion concentrations.

*Methods.* Into a series of Erlenmeyer flasks were placed in succession 5 cc. of a concentrated urea or ammonium carbonate solution, 10 cc. distilled water, 2 cc. of an indicator solution (phenol red brom-cresol purple or methyl red) and 2 cc. of an urease enzyme solution prepared according to the method of Folin and Youngburg.[1] The pH of successive flasks in a series was made to differ by 1 pH through addition of HCl or NaOH. The actual pH was determined by comparison with standard buffer mixtures to which the same indicators had been added. Any tendency for the pH to alter as a result of chemical interactions was compensated by adding small amounts of HCl or NaOH during the course of the experiment. The flasks so prepared for any set of observations were kept for a definite interval of time and at a constant temperature. In order to determine optimal conditions, the temperature in different sets of experiments was varied from that of the room to 45° C., and the reactions were allowed to continue from 15 min. to 2½ hours in different groups. It may be stated that the changes to be reported were obvious within 10-15 min. but usually became more pronounced after longer time intervals (45 min. to 1½ hours).

At the end of the time set, the amount of $(NH_4)_2CO_3$ formed in the urea solutions was determined by Nesslerization, compared with known standards and expressed in mg. ammonia nitrogen. Similarly, the amount of urea formed in the ammoniun carbonate solution was estimated by determining the *relative* quantities of unconverted $(NH_4)_2CO_3$ remaining in each flask. Unless it is assumed that $(NH_4)_2CO_3$ escaped as gas during the course of the experiment, which is not probable under the conditions of the experiment,* it is

[1] Folin, O., and Youngburg, G. E., *J. Biol. Chem.*, 1919, xxxviii, 111.

* This source of error is improbable in view of the facts (1) that the greatest reduction in the $NH_4$ nitrogen occurred toward the acid side, while a greater tendency to liberate $NH_4$ would exist toward the alkaline side, and (2) that control flasks in which boiled urease had been placed showed no alterations in the $NH_4$ nitrogen, when the pH was similarly altered.

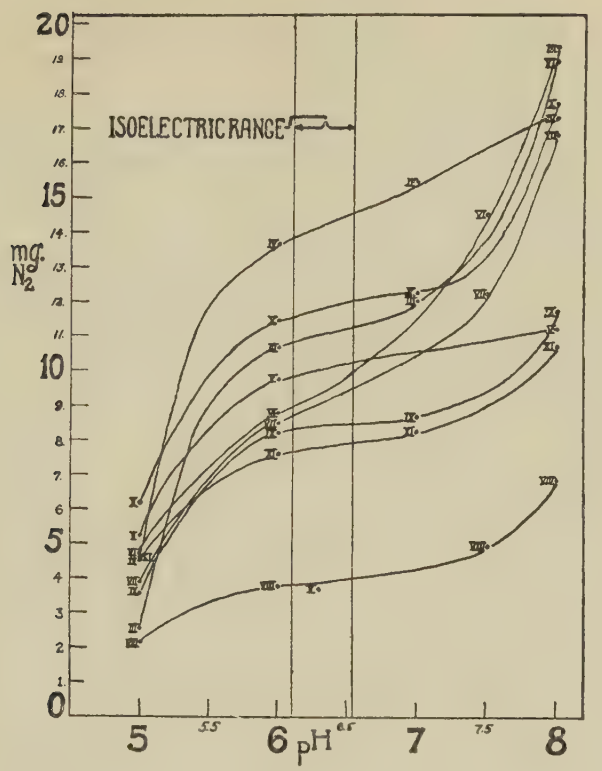


FIG. 1.

Curves showing mg. ammonia nitrogen (ordinates) obtained from action of urease on same initial quantities of urea at different pH (abscissae). Duration of action as follows: III, 75 min.; IV, 20 min.; VII, 15 min.; VIII, 6 min.; IX, 45 min.; X, 10 min.; XI, 5 min.

reasonable to assume that the relative losses of $(NH_4)_2CO_3$ are a criterion of the amount converted to urea. The actual presence of urea was demonstrated by use of the xanthydrol test. Thus, it was noted that the volume of precipitate formed agreed roughly with the loss of ammonia N. Quantitative estimates were not made however.

*Results.* The results of 9 experiments in which the effects of urease on urea solutions was tested are plotted in Fig. 1. They indicate that while $(NH_4)_2CO_3$ is formed from urea at all pH ranges, the conversion is slight toward the acid side of the isoelectric range and great toward the alkaline side. The curves of ammonia nitrogen so plotted also show the velocity of the reactions. They rise convex to the abscissae between pH 5-6, become horizontal

during the isoelectric range (6.1-6.5), established by Sumner[2] for crystalline urease, but rise rapidly and concave to the abscissae beyond this range.

The results of 7 experiments in which the effects of urease on $(NH_4)_2CO_3$ solutions were tested are graphically presented in Fig. 2. The curves showing the mg. of unconverted ammonia nitrogen and by inference the amount of urea formed at various pH points, demonstrate that a reversal of the curve contours from that of Fig. 1 obtains. They show also that urea formation gradually diminishes during the acid range; that it tends to become stabilized during the isoelectric range, but rapidly declines toward the alkaline side. The change in the inflection of the curves again occurs during the isoelectric range.

*Conclusion.* The results strongly support the probability that changes in pH can determine the directional action of the reversible

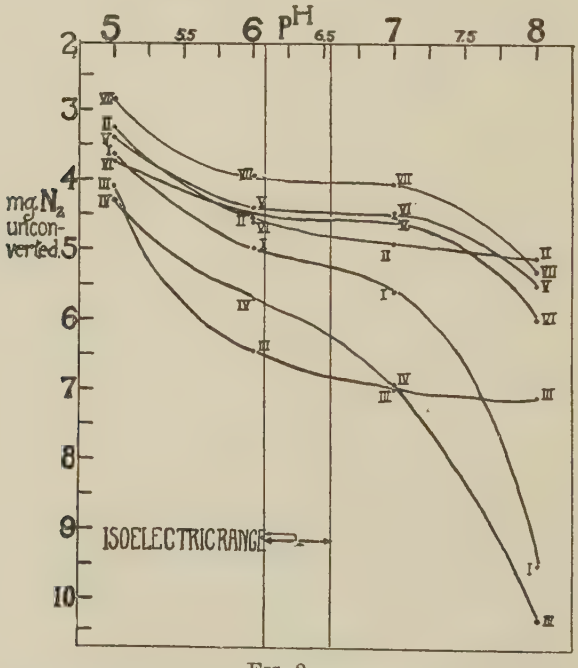


FIG. 2.

Curves showing mg. ammonia nitrogen unconverted to urea and by inference amount of urea formed (ordinates) after action of urease on same initial quantities of $(NH_4)_2\ CO_3$ at different pH (abscissae). Duration of action as follows: I, 47 min.; II, 120 min.; III, 60 min.; IV, 45 min.; V, 35 min.; VI, 70 min.; VII, 135 min.

[2] Sumner, J. B., and Hand, D. B., *J. Biol. Chem.*, 1928, lxxvi, 149.

ferment, urease. Toward the alkaline side of the isoelectric range the tendency to convert urea into $(NH_4)_2CO_3$ predominates; toward the acid side the tendency to synthesize urea is more pronounced.

In conclusion the writer wishes to express his gratitude to Dr. M. Garcia Banus for his advice and supervision of the work.

## 4697

## Exogenous Arginine as the Precursor of Creatine in the Dog.

R. LORIMER GRANT, ADAM A. CHRISTMAN AND HOWARD B. LEWIS.

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Of the substances normally present in the diet, structural relationships point to arginine as the most logical precursor of creatine and creatinine. In view of the recent work of Benedict and Osterberg,[1] who fed creatine over long periods of time, and the confirmation of their work in man by Rose, Helming and Ellis,[2] it seemed possible that earlier attempts to demonstrate that creatine may originate from exogenous arginine failed because the amino acid was administered over too short a period to observe any significant change in the excretion of creatine or creatinine. Therefore it was decided to study the effect of arginine on the creatine-creatinine elimination, when the arginine was fed daily to a dog over a period of several weeks. After the completion of the experiment, Hyde and Rose[3] published the results of a similar study of prolonged administration of arginine to a normal man and woman. Although their results showed no evidence of the conversion of exogenous arginine to creatine or creatinine, they have suggested that other species, particularly the pig,[4] may differ in their response to arginine feeding. Our results presented in Table I, with another species, the dog, confirm those of Hyde and Rose, in that oral administration of arginine for a period of 35 days failed to influence the excretion of urinary creatine or creatinine, although exogenous creatine (Periods XIII and XIV) in small amounts resulted in prompt increases in both these catabolites, thus confirming the earlier work of Benedict and Osterberg.

[1] Benedict, S. R., and Osterberg, E., *J. Biol. Chem.*, 1923, lvi, 229.

[2] Rose, W. C., Ellis, R. H., and Helming, O. C., *J. Biol. Chem.*, 1928, lxxvii, 171.

[3] Hyde, E. C., and Rose, W. C., *J. Biol. Chem.*, 1929, lxxxiv, 535.

[4] Gross, E. G., and Steenbock, H., *J. Biol. Chem.*, 1921, xlvii, 33.

Our experiment was carried out with a trained metabolism dog, female, of about 14 kg. weight. Urine was collected by catheterization daily and analyzed for creatine and creatinine by the standard micromethods of Folin. The initial basal diet consisted of cracker meal, 50 gm., casein, 20 gm., evaporated milk, 200 cc., sucrose, 25 gm., bone ash, 10 gm. and vegex (as a source of vitamin B), 5 gm. Unfortunately, after the first two periods, the animal began to refuse this diet, so that it was necessary to add 25 gm. of fresh beef heart. The meat was thoroughly ground and mixed, the daily rations weighed out in separate portions and kept frozen in a freezing chamber. The basal diet on analysis was shown to contain 6.36 gm. of nitrogen and was estimated to furnish 650 calories or about 46 calories per kilo of body weight.

TABLE I.

*Creatine-Creatinine Excretion in the Dog After the Feeding of Arginine and Creatine.**

| Period | Body weight | Dietary N | Urine | | | |
|---|---|---|---|---|---|---|
| | | | Total N | Creatinine | Creatine as Creatinine | |
| | kg. | gm. | gm. | mg. | mg. | |
| I | 14.2 | 5.61 | 3.57 | 386 | 23 | |
| II | 14.1 | 5.61 | 3.31 | 379 | 26 | Fore periods |
| III† | 14.0 | 6.36 | 4.55 | 387 | 29 | Basal diet. |
| IV | 14.1 | 6.36 | 5.35 | 400 | 13 | |
| V | 14.1 | 6.53 | 5.59 | 415 | 18 | 1 gm. arginine hydrochloride daily. |
| VI | 14.1 | 6.53 | 5.63 | 421 | 13 | 1 gm. arginine hydrochloride daily. |
| VII | 14.2 | 6.76 | 5.70 | 421 | 14 | 1.5 gm. arginine hydrochloride daily. |
| VIII | 14.3 | 6.76 | 5.51 | 419 | 20 | 1.5 gm. arginine hydrochloride daily. |
| IX | 14.1 | 6.89 | 6.06 | 424 | 13 | 2 gm. arginine hydrochloride daily. |
| X | 14.0 | 6.36 | 5.41 | 434 | 6 | After periods. |
| XI | 14.0 | 6.36 | 5.45 | 427 | 14 | |
| XII | 14.0 | 6.36 | 5.35 | 426 | 9 | Basal diet. |
| XIII | 14.0 | 6.57 | 5.31 | 453 | 49 | 0.75 gm. creatine hydrate daily. |
| XIV | 14.0 | 6.64 | 4.76 | 477 | 218 | 1 gm. creatine hydrate daily. |
| XV | 13.9 | 6.36 | 5.20 | 459 | 41 | After periods. |
| XVI | 13.9 | 6.36 | 4.92 | 438 | 20 | Basal diet. |

* The figures represent daily averages for the periods. All periods except II and IV (6 days each) were of 7 days' duration.

† Beginning with Period III, 25 gm. of beef heart daily were added to the basal diet.

A slight increase in the total creatinine was observed in Periods III to V, the periods immediately following the addition of the beef heart to the basal diet. We believe this is to be ascribed to the response of the metabolism to the small amount of creatine (estimated at 60 mg.) present in the beef heart.

Since administration of arginine did not alter the urinary creatine or creatinine and since the administration of creatine was followed by an increased urinary elimination of both creatine and creatinine (maximum values, 494 and 380 mg. of creatine and creatinine respectively on the 6th day of Period XIV), no evidence was obtained that under our experimental conditions, exogenous arginine had any relationship to the urinary creatine or creatinine in the dog.

## 4698

## The Growth-Promoting Power of Egg for Planarian Worms.

ROSALIND WULZEN.

*From the Department of Animal Biology, University of Oregon, Eugene, Ore.*

It has been previously demonstrated by us[1] that an exclusive diet of egg albumin is not capable of promoting growth in *Planaria maculata.* Although the food is well taken, the worms show progressive decrease in size throughout the experimental period. Upon an exclusive diet of egg yolk the worms may maintain or increase their size for a period of 2 or 3 weeks, but after that time their length becomes progressively less.

We have confirmed these results repeatedly with the species of planarian worm *(Planaria agilis?)* which we are at present using, and have found in addition that a combination of egg yolk with egg albumin produces a diet decidedly superior to either substance used alone.

Each group in an experimental series consisted of 30 worms of equivalent size which were fed *ad lib.* twice a week on the experimental diets. The total length of the worms was found at the beginning of the experiment and at its conclusion and the proportional increase in length was determined. In one experiment of 2 weeks duration kept at room temperature, the group of 30 worms fed upon egg yolk made a gain in total length of 3.9%, while those fed upon a combination of equal parts of egg yolk and albumin gained 20.2%

[1] Wulzen, R., *Univ. of Calif. Publications in Physiology,* 1923, v, 175.

in total length. In another series incubated at a temperature of 27° C. over a 2 week period, egg yolk diet produced an increase in total length of 32%, while a diet of blended egg yolk and albumin in equal parts gave an increase in total length of 51.5%. In a third series pastes were made of 15% liver pulp and 45% starch paste (10%) with the addition of 40% egg yolk or egg albumin or a mixture of equal parts egg yolk and albumin. The starch and liver pulp were added for purposes not connected with this paper. The worms were fed over a 3 week period and were kept incubated at a temperature of 23° C. Only a few of those fed upon albumin were able to survive the experimental period. The group of worms fed upon egg yolk gained 16.8% in total length, while those fed a mixture of yolk and albumin gained 68.1%.

It will be noted that in the experiments cited which were of 2 weeks duration a yolk-albumin mixture exceeded yolk alone in growth-promoting power by 16.3% and 19.5% respectively. In the 3 week experiment, the mixture of egg yolk and albumin in equal parts exceeded yolk in growth-promoting power by 51.3%. When albumin is fed alone a negative growth is obtained.

4699

## Body Fluid of the Mammalian Embryo as a Medium for Tissue Culture Work.

PETAR N. MARTINOVITCH. (Introduced by George A. Baitsell.)

*From the Centralni Higijenski Zavod, Belgrade, Serbia, and the Osborn Zoological Laboratory, Yale University.*

The exudates in the peritoneal cavity of the frog and guinea pig have recently been used[1] as culture media for cultures *in vitro*. In the present experiments, various tissues of the albino rat have been successfully cultivated in the peritoneal cavity exudate of the same animal. However, the growth of certain tissues, which it was particularly desired to cultivate was not so vigorous as hoped for. Consequently an endeavor was made to find a culture medium better adapted for the cultivation *in vitro* of these tissues.

Attention was centered upon a medium containing embryonic tissue juice because of the favorable results obtained by various in-

[1] Baitsell, G. A., and Sherwood, M. B., PROC. SOC. EXP. BIOL. AND MED., 1925, xxiii, 96.

vestigators with this type of medium. It was felt, however, that the usual method of obtaining the embryonic tissue juice by mincing the embryonic tissues in a saline solution might be improved upon by using body fluids normally present in the embryo. There was the possibility that these fluids might contain the growth promoting substances and thus obviate the necessity of adding embryonic tissue juice to them.

In working with newborn albino rats it was observed that a clear fluid would flow from little cuts in the skin, and it was decided to try this exudate. However, there was considerable difficulty in getting a sufficient supply for use as a culture medium. It was found that a much more abundant supply of body fluid could be obtained from the embryo by inserting the point of a fine bore pipette through the skin and frontal bone into the underlying cranial cavity. Care should be taken not to insert the point of the pipette too deeply or the brain tissue may be disturbed and bits of it be taken up into the pipette. However this material, as well as blood cells, can easily be removed by centrifuging. If the point of the pipette is in the proper position, a clear fluid flows into it as soon as one releases the pressure on the bulb. It was later found that the best results were noted when the fluid was obtained from foetuses between 18 and 20 days of age, although somewhat younger embryos may also be used. Fluid sufficient to make several cultures can usually be obtained from one embryo.

Tissues from several organs, notably, brain, ependyma, liver, spleen, testis, intestine, as well as striated muscle tissue and connective tissue from various regions, were cultivated in this embryonic body fluid culture medium with excellent results. It was seldom that the explant did not show some signs of growth, and in many cultures very large growths were obtained. Explants of ependyma, liver, spleen, and connective tissues from the leg, when taken from embryos 19 or 20 days of age and supplied with body fluid from embryos of the same age, gave the best results. The first signs of growth are, in some cases, to be observed after only a few hours of incubation. Cultures which show no activity by the end of the first 24 hours rarely show any growth later. After 3 days of incubation, active growth in the cultures usually stops, and it is necessary to make subcultures and supply fresh medium to insure continued activity.

Some experimental evidence has been obtained indicating that the body fluid as found in the different regions may show a certain amount of specificity for the tissues with which it comes into direct

contact when in its natural position. Thus it was observed in a number of cases, that when the body fluid obtained from the cranial cavity, as described above, is used, the first cultures to show activity are those containing explants of the tissue lining the cranium.

My thanks are due to Dr. R. G. Harrison for the laboratory facilities at Yale; to Dr. G. A. Baitsell, at whose suggestion the experiments were started; and to Dr. J. S. Nicholas for supplying the animals that were used in these experiments.

4700

## Acute Dilatation of the Stomach.*

LESTER R. DRAGSTEDT AND JAMES C. ELLIS.

*From the Department of Surgery of the University of Chicago.*

In a previous communication we[1] have reported that the total loss of gastric juice from the body causes death in the dog in from 5 to 10 days, with symptoms of depression and marked changes in the blood chemistry. These may be summarized as follows: a progressive decrease in the concentration of blood chloride, an increase in the $CO_2$ capacity, an increase in the pH, and a late increase in the N P N and Urea N. Water and salts are not absorbed to any appreciable extent in the stomach or duodenum and it is therefore clear that any factor such as obstruction at the pylorus or in the duodenum, gastric or duodenal fistula, or profuse persistent vomiting, will lead to this loss of gastric juice constituents through failure of reabsorption in the lower intestine. It is likely that the property of the gastric mucosa, by virtue of which it can continue to separate the elements for its secretion from the altered blood plasma until death is produced, is of major importance in the pathogenesis of these disorders. In the present communication we wish to report a case of acute post-operative dilatation of the stomach together with evidence which indicates that here also the failure of reabsorption of gastric juice is the cause of death.

The patient, a female of 70, was brought to the hospital with a strangulated femoral hernia. The findings are unimportant for the

* This work has been conducted under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

1 Dragstedt, L. R., and Ellis, J. C., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 305.

purpose of this paper except for the blood chemistry report, which is as follows: Chloride 196 mg., $CO_2$ 60 cc.; Urea N 14.0 mg., and N P N 31 mg.

There had been no vomiting. A blood transfusion (500 cc. citrated blood) was given and a section of gangrenous ileum, imprisoned in the hernial sac, resected. 3000 cc. of Ringer's solution was given by vein daily for the next 5 days but the patient gradually became weaker and changes in the blood chemistry more marked. On the 6th day the blood chloride had decreased to 180 mg., $CO_2$ capacity 86 cc., N P N 46 mg., and Urea N 32 mg. On the 9th day the patient vomited 60 cc. of brownish fluid, containing no free acid and a total chloride concentration of 0.35%. Gastric lavage had not been done because of auricular fibrillation. The daily intravenous injection of Ringer's solution was continued, but in spite of this the blood chloride continued to fall and on the 10th day reached 156 mg. Death occurred on the 12th day. Autopsy revealed an enormously dilated stomach, reaching to the pelvis, and containing about 3 liters of thin brown fluid. Unfortunately this was lost, but it is probable that its composition was similar to the vomitus secured on the 9th day. A carcinoma of the neck of the gall bladder, extending to and producing stenosis of the pylorus was the other significant finding.

This case lends support to the view expressed in 1922[2] that the cause of acute dilatation of the stomach is reflex inhibition of the gastric motor mechanism through the vagi and splanchnics as a result of the operative trauma which produces intense stimulation of visceral sensory nerves. As a consequence of this extreme relaxation, gastric juice could not be forced through the narrowed pylorus and was lost to the body either through accumulation in the stomach or vomiting. Whereas in this case the important factor was the failure of reabsorption of gastric juice in the majority of cases of acute dilatation, uncomplicated by pyloric stenosis the loss of pancreatic juice and bile probably also occurs because of the secondary mechanical occlusion of the inferior horizontal portion of the duodenum by the mesentery of the small intestine.

[2] Dragstedt, L. R., and Dragstedt, C. A., *J. Am. Med. Assn.*, 1922, lxxix, 612.

4701

## The Action of Specific Diuretics: The Inhibiting Effect of Intraperitoneal Distilled Water.

GEORGE M. CURTIS.

*From the Department of Surgery, University of Chicago.*

Administration of theophylline ethylenediamine intramuscularly to rabbits calls forth an acute and profuse diuresis. In an extensive physico-chemical study of the initiating events, as well as of the subsequent diuresis, it was found that a slight but constant rise in the whole blood chloride occurs relatively soon after the administration, and as a rule precedes the maximal diuresis. The resulting diuresis may exceed 50 times the normal urinary output. The specific electric conductivity of the urine and particularly the concentration of the urinary chloride rise, even throughout the periods of increasing and maximum diuresis. Consequently so much as one third the amount of the total blood chloride may be excreted within an hour. However, at the end of the hour the whole blood chloride is even higher than during the control preadministration period. It follows that during the hour chlorides pass rapidly from the tissues into the blood stream, and thence through the kidney into the urine.

A consideration of these findings, the rise in blood chloride and the profuse excretion of urinary chloride, led to a consideration of the importance of the chloride in the mechanism of the induced diuresis. Cohnheim[1] had found that chloride soon passes into sugar solutions injected intraperitoneally. The intraperitoneal injection of sugar solutions results in a fall in the blood chloride, since chloride leaves the blood stream and enters the artificial transudate up to a concentration of 0.4%.[2] Other electrolytes are likewise deviated from the blood stream, after the laws governing permeability. When hypotonic sugar solution is injected intraperitoneally at the same time that the specific diuretic is administered intramuscularly, there follows a definite decrease of the diuretic response. This decrease is not so marked when isotonic sugar solutions are injected. The diuresis which remains is associated with an increased excretion of sugar. A similar diuresis follows the injection of the sugar solutions alone. The substitution of isotonic saline in a combined injection has no inhibiting effect, in fact the resulting diuresis is unusually large.

[1] Cohnheim, O., *Z. f. Biol.*, 1899, xxxvii, 443.

[2] Curtis, G. M., *Biochem. Z.*, 1925, clxiii, 109.

When distilled water is injected intraperitoneally at the same time the diuretic is administered intramuscularly no diuresis ensues.[3] The normal action of the specific diuretic is thus inhibited. There are no crystalloids to enter the blood. Also as high as 0.63% sodium chloride together with other electrolytes are rapidly deviated from the blood stream and tissues. There is a distinct fall in the blood chloride. At the end of an hour a secondary diuresis may ensue, and at that time a subsequent injection of the diuretic may become effective. However, then the peritoneal cavity contains an isosmotic fluid with about 0.6% sodium chloride. The same inhibitory effect occurs after the denervation of the kidneys.[4]

These experimental findings led to the conclusion that the deviation of chlorides and other electrolytes from the blood stream and tissues, and consequently from the kidney, was the important factor in the inhibition of the normal action of the specific diuretic employed. Objection has been made to this view on the basis that the intraperitoneal distilled water may produce some acute change in the freely exposed kidney, such as an acute edema, and that this is the factor inhibiting renal secretion. In the rabbit, to be sure, the kidney hangs nearly free within the peritoneal cavity, and is covered by but a thin serous layer, with some properitoneal fat. The present series of experiments was consequently designed to answer this and other similar questions.

In 15 experiments distilled water at body temperature was perfused through the peritoneal cavity at the rate of 500 cc. per hour. Within 15 minutes after the beginning of the perfusion there is, as a rule, a prompt decrease in the urinary output. Within an hour this often amounts to a complete anuria. In certain experiments there is a slight, irregular urinary secretion; however, this is consistently less than normal. In 5 of the experiments the diuretic was administered, 0.12 gm. intramuscularly, during this period of anuria or of lessened urinary secretion, from one-half to 2 hours after commencement of the perfusion. In no experiment was there an increased urinary output. During the perfusion chloride and other electrolytes are dialyzed away from the blood stream. Organic crystalloids, particularly glucose, also appear in the perfusion water. The blood chloride falls as low as 118 mgm. per 100 cc. From 300 to 836 mgm. of chloride is dialyzed from the blood stream and particularly the tissues.

In 15 experiments 0.12% glucose was added to the perfusion

[3] Curtis, G. M., *Biochem. Z.*, 1927, clxxxvi, 95.

[4] Curtis, G. M., and Shambaugh, N. F., *Biochem. Z.*, 1927, clxxxvi, 112.

water. The inhibiting effect upon the secretion of the urine is similar, though not so marked. The blood chloride falls as low as 143 mgm. per 100 cc. and the amount of chloride dialyzed away varies from 205 to 896 mgm. Administration of the specific diuretic during the perfusion is again without effect in increasing the urinary output. The similar perfusion of Ringer's solution, or of 0.9% sodium chloride, through the peritoneal cavity does not inhibit the urinary output; on the contrary it increases it.

The timed intravenous administration of salt solution of varying concentrations by means of the Woodyatt pump results in a diuresis. Consequently, an attempt was made to test renal function during the intraperitoneal perfusion of distilled water by simultaneously injecting timed adequate saline intravenously. In the 30 experiments during which either distilled water or distilled water with 0.12% glucose was perfused through the peritoneal cavity the amount of chloride dialyzed away from the bloodstream and tissues varied between 205 and 896 mgm. An effort was consequently made to supply pure sodium chloride in timed intravenous amounts to the blood stream at least as rapidly as it would be dialyzed away by the perfusion water. After calculation and a number of trials it was found best to give 2.5% sodium chloride intravenously at the rate of 1 cc. per minute during the time that distilled water was being perfused through the peritoneal cavity at the rate of 500 cc. per hour.

When this simultaneous perfusion of distilled water and intravenous injection of adequate saline is effected, kidney function is not inhibited and urinary secretion does not decrease. On the contrary it increases, even markedly. In 4 such experiments there was a definite increase in urine formation; in one as long as 13½ hours following the commencement of the simultaneous perfusion of distilled water intraperitoneally. In another experiment urinary secretion was resumed, after an anuria, upon starting the pump one hour after the transperitoneal perfusion began.

These experiments demonstrate that perfusion of distilled water through the peritoneal cavity does not inhibit kidney activity and the secretion of the urine by direct action upon the kidney cells through the thin peritoneal covering. The question of an associated acute edema of the kidney may be reasonably answered in the negative. If adequate salt is simultaneously injected intravenously, kidney function may be maintained for hours during the perfusion of distilled water through the peritoneal cavity. The conclusion that the inhibitory effect of intraperitoneal distilled water upon the action of the specific diuretic is due to deviation of chloride and other electrolytes from the blood stream and tissues is still reasonably warranted.

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## Types of Disturbances of Mineral Metabolism Associated With Bone Dystrophies.

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In addition to rickets arising from diets deficient in vitamin D or salts of calcium or phosphorus, bone dystrophy has been noted in various disturbances of mineral metabolism which lead to imperfect deposition of the mineral constituents of bone, or to their excessive mobilization. Because of the similarity of clinical picture and the mechanics of production, the term rickets may justifiably be applied to the whole of this diverse group of bone dystrophies. Those cases arising from deficient absorption of inorganic bone constituents should be considered of exogenous origin, whether the deficient absorption be due to inadequate intake of minerals or of vitamin D, to inadequate ultraviolet irradiation, or to other cause. Those instances in which the metabolic disturbance is not dependent upon inadequate absorption are secondary to some endogenous disturbance of metabolism.

Several patients with endogenous rickets have been studied intensively in this clinic, and while their bone lesions were very similar, the metabolic data indicate that their anomalies were not dependent upon a common metabolic defect. The nature of the metabolic data indicated that in one group of patients the condition was secondary to chronic acidosis, due in one instance to persistent ketosis, and in another to constant reabsorption of urine. In a second group the bone changes were dependent upon hyperparathyroidism. The remaining group must be termed idiopathic, until further studies serve to establish the etiology of their condition. This third group is very similar in some respects to infantile rickets of the usual, or exogenous, type; it is distinguished from it in its failure to respond satisfactorily to adequate calcium and phosphorus ingestion, to vitamin D in the form of cod liver oil or viosterol, or to ultraviolet irradiation.

The accompanying table presents a correlation of the nature of the changes in the metabolism of phosphorus and calcium observed in this clinic, together with data collected from the literature.

TABLE I.—*Bone Dystrophies of Metabolic Origin.*

| Classification | Remarks | Metabolic Manifestations | | | | | |
|---|---|---|---|---|---|---|---|
| | | Serum* | | Urine† | | Feces† | |
| | | Calcium | Phosphorus | Calcium | Phosphorus | Calcium | Phosphorus |
| I. Exogenous | | | | | | | |
| Infantile rickets | Due to deficiency of Ca, P, vitamin D, or ultraviolet light | 10-12 mg. | 1-4 mg. | low | normal | high | high |
| Starvation osteomalacia[1] | | 5-7 mg. | 1-3 mg. | low or normal | low or normal | high | high |
| II. Endogenous | | | | | | | |
| a. Endocrine dysfunction | | | | | | | |
| Hyperparathyroidism[2] | Parathyroid adenoma; diffuse rarefaction, cyst formation | 10-18 mg. | 1-3.5 mg. | high | high | normal or high | normal or high |
| Hyperthyroidism[3] | Exophthalmic goiter, toxic adenoma; diffuse rarefaction | 9-12 mg. | 3-4 mg. | high | high | normal or high | normal or high |
| b. Chronic Acidosis | | | | | | | |
| Renal Insufficiency[4] | "Renal rickets"; deficient calcification, diffuse rarefaction | 5-9 mg. | 6-17 mg. | low | low | normal or high | high |
| Ureteral Transplantation[5] | Base deficit acidosis; deficient calcification | 10-12 mg. | 2-4 mg. | low | | | |
| Chronic Ketosis[6] | Atypical diabetes; deficient calcification and diffuse rarefaction | 8.6-11 mg. | 1.5-4 mg. | ‡ | ‡ | ‡ | ‡ |
| c. Idiopathic[7] | Similar to "exogenous" group, but do not respond to adequate diet and ultraviolet light | 10-12 mg. | 1-4 mg. | low | normal or high | high | high |

* These values do not necessarily cover absolute range of variation, but are quoted from literature or authors' data.

† Absolute values would be dependent upon intake, and so are not quoted.

‡ Urine-feces partition impossible.

[1] Miles, L. M., and Feng, C. T., *J. Exp. Med.*, 1925, xli, 137.

[2] Boyd, J. D., Milgram, J. E., and Stearns, G., *J. Am. Med. Assn.*, 1929, xciii, 684. Gold, E., *Mitteil. a. d. Grenzgeb. d. Med. u. Chir.*, 1928, xli, 63. Barr, D. P., Bulger, H. A., and Dixon, H. H., *J. Am. Med. Assn.*, 1929, xcii, 951. Wilder, R. M., *Endocrinol.*, 1929, xiii, 231.

[3] Aub, J. C., Bauer, W., Heath, C., and Ropes, M., *J. Clin. Invest.*, 1929, vii, 97.

[4] Schoenthal, L., and Burpee, C., *Am. J. Dis. Child.*, (in press).

[5] Boyd, J. D., PROC. SOC. EXP. BIOL. AND MED., 1928, xxvi, 181.

[6] Authors' data, unpublished.

[7] Authors' data, unpublished.